◎分子標的治療薬

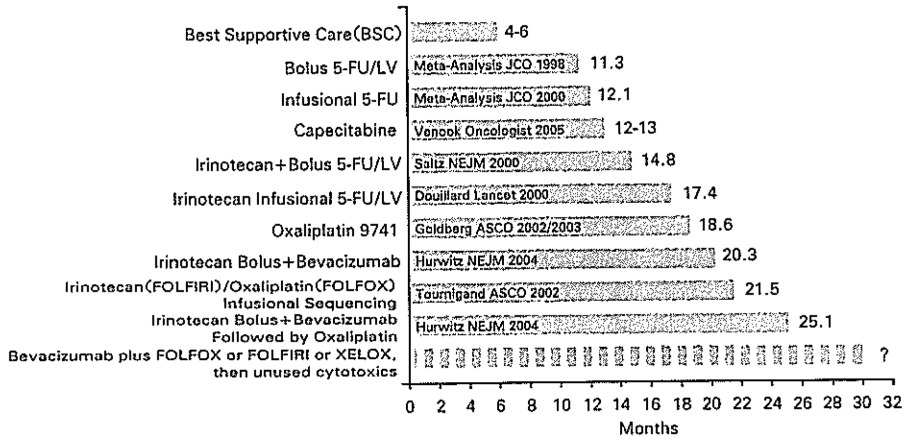
近年，新しい発想の薬物が登場しつつあります。ヒトゲノムの解明に伴い，細胞の細かい働きが徐々に解明されてきました。そうした研究からがんに特殊なものを細胞レベル・分子レベルで捉え，その部分にのみ作用する薬を開発するということが盛んに行われています。こうした発想で作られた薬物を「分子標的治療薬」と呼んでいます。

正常の細胞であれば，皮膚なら皮膚の，腸粘膜であれば腸粘膜の特性に則して，秩序正しく分化し，増えていきます。がん細胞は，そうした正常細胞が変異することで，正常細胞と異なる異常な分化・増殖をするわけですから，そうしたがん細胞特有の異常なシステムを細胞・分子レベルで解明し，そこをターゲットにすることにより，がん細胞を死滅させたり，増殖できないようにするのが分子標的薬です。

こうした研究が発展することで，化学療法にも「テーラーメイド」の治療が可能になります。ある患者さんのがん組織をDNA解析すると，そのパターンがたとえば「パターン3」だったとします。投与可能な薬剤がA，B，C，D，Eとある場合，「パターン3であれば，BとEという薬剤の組み合わせに感受性がある，すなわち効果がある」ということになれば，それを選んで患者さんに投与することが可能です。

現時点では，個々の患者さんが持つがん組織が，ある抗がん剤に対して明らかに感受性があるのか，ないのかが明確にならない状態で，その抗がん剤を投与せざるを得ない側面があります。結果として，化学療法が有効な患者さんもあれば，効果を示さない患者さんもあるということになります。しかし，その患者さん個々のがん細胞の違いは何なのかが事前に分かれば，無意味であるばかりか，副作用まで生じてしまうような化学療法を事前に知ることができ，回避することが可能となります。

分子標的治療薬のひとつの例として，「ハーセプチン」があります。細胞には，ある信号をキャッチすることで，「増えろ」と命令が与えられるような仕組みがあり，その「キャッチする」部分を「受容体（レセプター）」と呼ぶのですが，ある種の乳がんの細胞には，そのレセプターが大量に存在し，結果として正常細胞に比して増殖しやすい性格の細胞となってしまいます。ハーセプチンは，その受容体に「合い鍵」のようなかたちで先回りして入り込み，それを塞いでしまうような作用をするのです。それまでの抗がん剤にはない発想で開発されたもので，かなり期待も高く，実際ハーセプチンにより転移が消失し



Adapted from Grothey A, et al. J Clin Oncol. 2004;22:1209-1214, and Venook A. Oncologist. 2005;10:250-261.

図８　多剤併用抗がん剤治療の効果：Overall Survival (Months)

てしまうような効果を示した患者さんもおられます。

このハーセプチンの治療を行う中で，どのような患者さんの乳がんにはこの薬の効果があり，どのような患者さんには効果が低いのかも分かってきました。これまで乳がんに対してはホルモン療法（抗がん剤とは異なりますが，これもお薬による治療です）が効果を示す場合が多く，このホルモン感受性の高い乳がんでは治療の余地があったのですが，これが効果を示さない場合はお手上げに近い状況だったのです。ところがハーセプチンは，ホルモン感受性が低く，「HER2 タンパク」と呼ばれるものの感受性が高いという性格の乳がんに効果を示すことが分かってきました。これまでは手の打ちようがなかった，ホルモン療法が効かない乳がんに対して効果を示す薬が登場したことは，患者さんにとって大きな朗報であると思います。

●多剤併用抗がん剤治療

化学療法は，多くの場合，手術ができないような進行がんで適応となるものです。図８は，進行した大腸がんに対する抗がん剤の治療成績（生存率）をグラフにしたもので，かつては進行大腸がんの患者さんは４～６カ月くらいしか存命していませんでしたが，2004 年では 25 カ月存命しています。もちろん平均ですから，中には「ほとんど治った」という方もおられますし，「数カ月しか生存できなかった」という方も含まれます。この成績が十分なものとは考

えておりませんが，さまざまな抗がん剤を組み合わせることにより，徐々に抗がん剤の治療成績も向上しています。これにお話した分子標的薬が加わることで，さらに患者さんが元気にしていられる時間が増やせるのではないかと考えられ，いまもさまざまな治療法・投与法が検討されています。

◎免疫療法

がんという病気に対しては，ヒトにそもそも備わっている「免疫力」を活用することが有効ではないかと考えられますから，免疫療法もさまざまなものが検討されてきました。

ヒトの免疫機能を強くする方法として，免疫を担う細胞（免疫担当細胞）を活性化するということが，種々のものについて検討されてきましたが，そのうち「樹状細胞」と呼ばれるものが免疫機能の鍵となる司令塔細胞なのではないかということで，研究が進んでいます。現時点では，樹状細胞そのものの活性化だけでは効果が弱いことが分かっており，現在，司令塔としての機能を十分に活用するため，免疫担当細胞の力をさらに増強させるべく基礎研究が進められています。

また，免疫療法のひとつとして「造血幹細胞移植」があります。幹細胞移植（骨髄移植）は，血液のがん（白血病）や悪性リンパ腫に対する治療法として行われてきましたが，その副作用として「移植片対宿主病（GVHD)」が問題となっていました。他人の骨髄を移植することで，免疫反応により皮膚にブツブツやただれが生じたり，下痢をするといった症状が生じます。ところがこうしたGVHDがある程度生じた患者さんで，むしろ白血病などのがんに対する治療効果が高いことが分かってきました。そこで，これまで副作用と考えられていたこの免疫反応を，むしろ治療に利用できるのではないかという発想で検討が進みました。

従来の幹細胞移植は，放射線治療や大量化学療法を伴う，患者さんにとってはかなりきつい治療方法で，高齢者では行えないような治療でした。しかし，免疫反応の効果に着目することで，そうした大量の抗がん剤を用いないやり方でも有効なのではないかと考えられ，「軽い幹細胞移植」という意味で「ミニ移植」と呼ばれ，実際に治療を行っています。当初はやはり幹細胞移植が高い効果を示す血液がんが対象の中心でしたが，腎臓がんや大腸がんが肺に転移したというような「固形がん」の患者さんに対しても効果があることが分かり，現在さまざまな方法が検討されています。

表1　国立がんセンターがん予防・検診研究センターでのがん検出率（2004. 2. 1〜2005. 1. 31）

	がん症例数	検査件数	がん検出率
食道がん	7	3730	0.19%
胃がん	35	3730	0.94%
大腸がん	46	3797	1.21%
肺がん	31	3764	0.82%
乳がん	14	1714	0.82%
前立腺がん	24	2044	1.17%
その他*	34	3730	0.91%
合計*	191	3792	5.04%

*：肉腫を含む

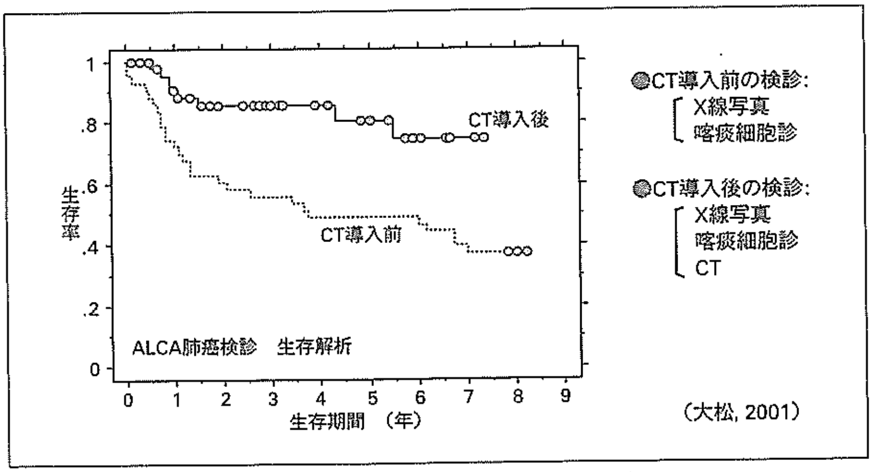


図9　CT検診で発見された肺がん症例の生存曲線
（「東京から肺がんをなくす会」の，CT導入前後の比較）

§4　がんに勝つには？〜早期診断のための機器の進歩

以上，がんの治療法についてのさまざまな進歩についてお話してきましたが，やはり進行がんの状況ではどうしても治療法が制限され，治癒が困難になります。しかし，早期がんに対する成績がかなり向上してきたことから，「がんの早期診断」の重要性がより高まり，現在がんセンターでも力を入れている分野となっています。

2年前（2004年），国立がんセンターに「がん予防・検診研究センター」がで

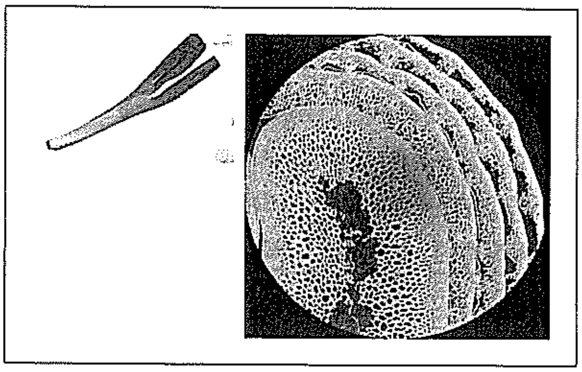


図10　顕微鏡CTによるネギの画像

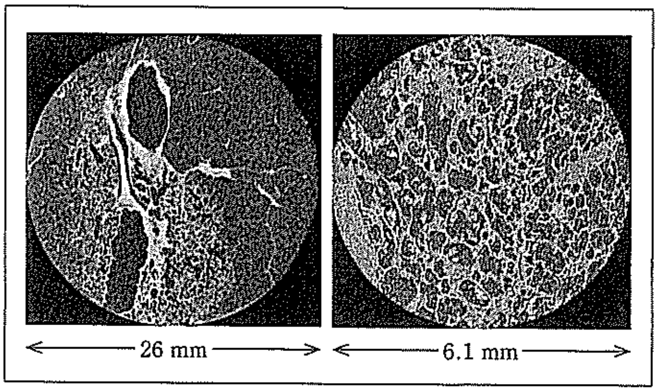


図11　肺がんの顕微鏡CT像

き，「いかに早期にがんを見つけるか」についての研究を行っています。そこでは，実際に検診を行って，がん検診の精度についての評価を行っています。表1は，センター設立の2004年2月から2005年1月までの1年間での，3,792名の方を検診させていただいた結果ですが，なんとそのうちの5%の方でがんが見つかっています。受診者の年齢などの理由もあるかも知れませんが，医療者としては，まだまだがんがあるのにそれが発見されないでいる人たちが，世の中に多いのではないか，という不安を強くおぼえる結果です。

がんの早期発見，がん検診については，診断器機についてもどんどん新しいものが開発されていますので，そのいくつかをご紹介します。

“CT（Computer Tomography)”という言葉もかなり一般的となりましたが，それがさらに進んだものに「ヘリカルCT」があります。「ヘリカル」というのは「らせん」という意味で，受診者のからだの回りをグルグル回ることで，より細密に画像データを収集できます。その結果，普通のレントゲンでは分か

表2　がんに負けないための秘訣

1. 生活習慣に気をつけましょう。
2. 検診を受けて早期発見，早期治療。
3. がんになったら，自分のがんの勉強をしましょう。
4. 担当医の話を良く聞き，分からないところは質問しましょう。
5. そして納得の行く治療を受けましょう。

らないような小さながんを見つけることや，そのデータをコンピュータで計算し，肺なら肺の立体的な画像（3D画像）を作ることも可能になります。**図9**は検診にCTを導入する前と後での，発見された肺がん患者の生存率を比較したものですが，大きな差が出ています。これはすなわち，治療で治すことができるような小さな肺がん，早期の肺がんがCTで発見されたことによります。

さらにCTは発展しており，「顕微鏡CT」というものも登場しました。がんを治療する上で，そのがんの性格，組織型を知ることが重要なのですが，そのためには実際に患者さんのがんの細胞を採って診断する必要があります。病巣に針を刺すようなことが必要ですから，患者さんにとって大きな苦痛となる検査です。顕微鏡CTは，そうした問題をクリアすべく研究が進められているものです。**図10**は，皆さんがイメージしやすいように，ネギを顕微鏡CTで撮影したものです。**図11**は肺の組織で，左は約10倍，右は約30倍程度の拡大像です。図左では，正常な肺では空気が入っているところ（肺胞）はつぶれていない一方，がんの部位ではそれが硬くなってしまい，がん細胞が周囲に浸潤していることが分かります。図右では，肺胞の中に向かってがんが増殖していることが分かります。こうした技術がさらに進歩すると，肺に針を刺し組織を採る，というような検査が不要になるのではないかと考えています。

おわりに〜がんに負けないための秘訣

最後に私が考える「がんに負けないための秘訣」を表2にまとめました。

まず「生活習慣に気をつけよう」。がんは生活習慣病であり，とくに喫煙ががん発生原因の1/3を占めることも皆さんよくご存じだと思います。黄緑色野菜をたくさん取るなど，普段の生活で健康に気をつけましょう。

そして「検診を受けて早期発見，早期治療」。今日のお話でその意義は十分

ご理解いただけたと思います。

がんという病気になってしまったら，「自分のがんについて勉強をしましょう」。そして「担当医の話をよく聞き，分からないことは質問しましょう」。その上で自分で考え，「納得いく治療を受けましょう」。

これが本日の皆さまへの最後のメッセージです。築地の国立がんセンターの周囲には，素晴らしいところがたくさんあります。お時間があったら，どうぞ見学においでください。

【参考資料】

〔資料 1〕財団法人がん研究振興財団：がんとどう付き合うか「大腸がん」予防と診断・治療・社会復帰と緩和ケア．

〔資料 2〕厚生省がん研究助成金，厚生省がん克服 10 か年戦略：がん—厚生科学の挑戦．

【シリーズ『がん医療の現在』バックナンバーから】

今回の講演では，さまざまな分野についてお話しましたが，各テーマについてさらに詳しく知りたい方は，本講演会シリーズの記録である『がん医療の現在（いま）』（医事出版社）のバックナンバーをご参照ください。以下に，近刊を中心に，今回のテーマにかかわる主なものを挙げておきます。

◎ がん発生のメカニズムについて：
- 廣橋説雄「がん研究の現状と展望」，がん医療の現在 No. 11（2004 年）所収．〔資料 3〕
- 若林敬二「がんの発生要因と予防方法」，がん医療の現在 No. 13（2005 年）所収．〔資料 4〕

◎ 内視鏡的治療について：
- 齊藤大三「消化管がん（食道がん・胃がん・大腸がん）の内視鏡的治療」，がん医療の現在 No. 10（2004 年）所収．

◎ 化学療法，分子標的治療薬について：
- 西尾和人「がんの分子標的治療～新しいがん治療の開発動向」，がん医療の現在 No. 9（2003 年）所収．
- 田村友秀「がんの化学療法（抗がん剤による治療）～肺がんを中心に」，がん医療の現在 No. 14（2006 年）所収．

◎ 免疫療法について：
- 高上洋一「がんの免疫療法と造血幹細胞移植」，がん医療の現在 No. 12（2005 年）所収．

◎ がん検診について：
- 祖父江友孝「がん予防・検診の最新情報～どのような検診をどのように受けるべきか」，がん医療の現在 No. 12（2005 年）所収．
- 森山紀之「がん検診でどこまでわかるか～がん予防・検診研究センターの役わりと PET」，がん医療の現在 No. 14（2006 年）所収．

Q&A

Q “PET” の検診精度はいかがでしょうか。

A “PET” については，前回（第 19 回）の本市民公開講演会で，予防・検診センター長の森山先生が詳しくお話されましたので今回は触れませんでした。PET でのがんの発見率は，やはりがんの進行度や種類などに影響されます。本日は肺のヘリカル CT についてご説明しましたが，たとえばごくごく早期の肺がんについては，PET よりヘリカル CT のほうが検診精度は高いということは言えます。しかし，診断精度の「評価」は，必ずしも「より小さいがんが見つかること」でなされるのではなく，「そのがんが見つかることで治療成績が改善されること」にあります。ですから，仮に肺がんに対する PET の診断精度がヘリカル CT に劣ったとしても，それでも十分治療が可能である程度に精度が高ければ，PET 検査を受ければそれで十分である，という判断もできます。

診断技術の進歩によって，以前では考えられないくらい小さながんが見つかるようになってきましたが，果たしてそれが間違いなく生命に危険を及ぼすようながんにまで進行するのか，ひょっとしたら消えてしまうものもあるのではないか，という問いに対して十分回答できるほどのデータが現時点ではないのです。危険性の少ないがんを治療することで，患者さんに余計な侵襲を加えてしまう可能性も否定できません。ですから，予防・検診研究センターではそうしたデータをたくさん集め，研究しているのです。

PET はオールラウンドな検査であり，その利用価値は計り知れないものがあります。PET で見つからないがんがあるということが，すなわち PET が不要であるという結論にはなりません。

Q 日本のがん医療も，世界の流れである放射線治療をもっと進める努力が必要ではないかと思います。

A ご指摘の通り，日本のがん医療は外科が優勢で，その他の治療法が少し遅れをとっているところはあります。国，行政のレベルでも，現在，放射線の治療専門医を何とかして増やそうと努力しています。先ほど池田先生も触れられましたが，各施設の放射線治療の管理が精度高く行われているのかを第三者的視点からチェックするような試みもその一環と言え，もう少し時間をいただければ，放射線治療もどんどん普及していくのではないかと思っています。そのように放射線治療の力の認識が広まっていけば，若い医師もそれに刺激され，高いモチベーション，インセンティブをもってその専門医を目指す人も増えていくと思います。放射線治療に限らず，化学療法等にしても，これから専門医をどんどん増やしていかなければなりません。

（編集部注：今回の講演の主旨と異なる質疑応答については割愛させていただきました。）

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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.

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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

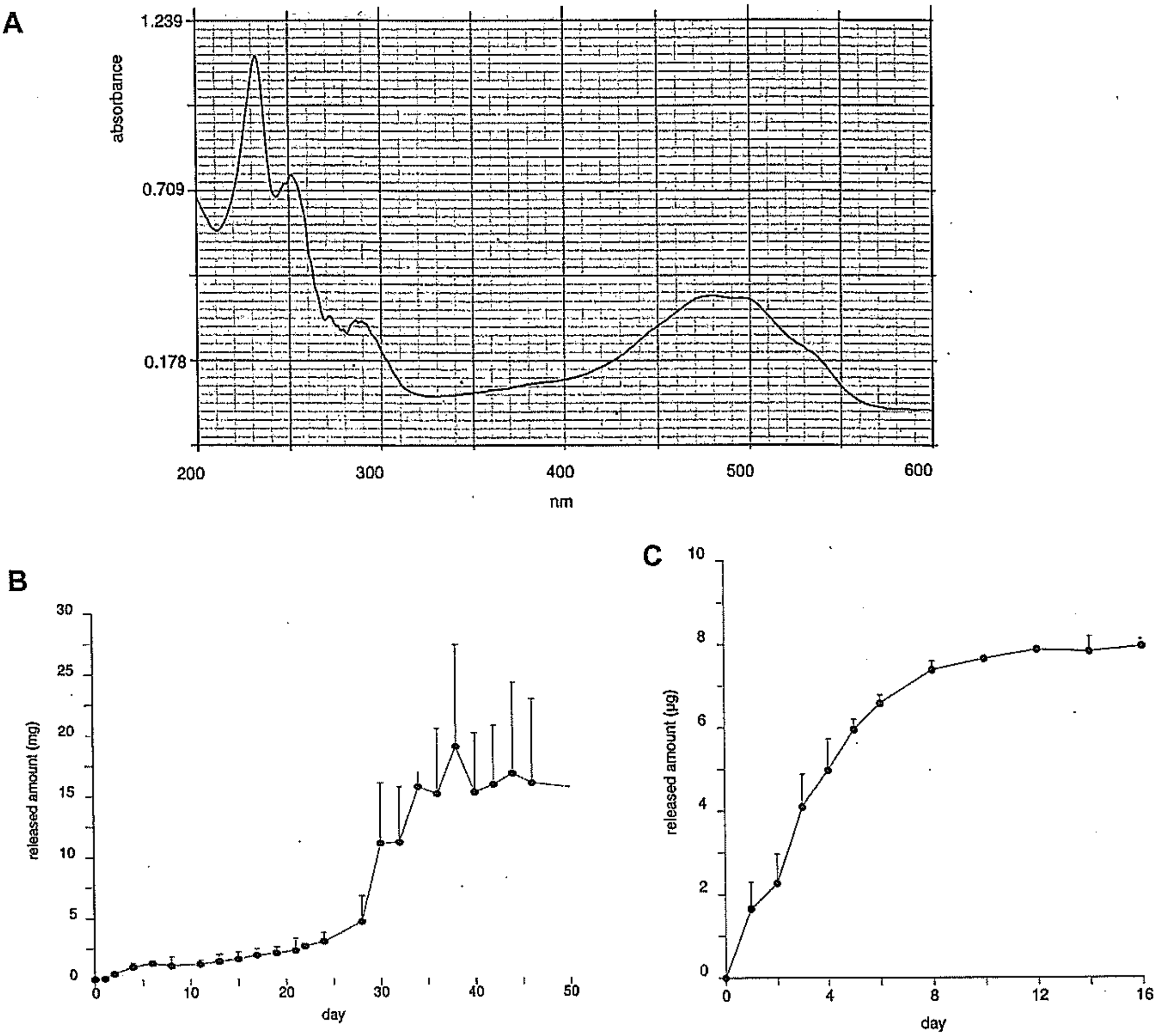
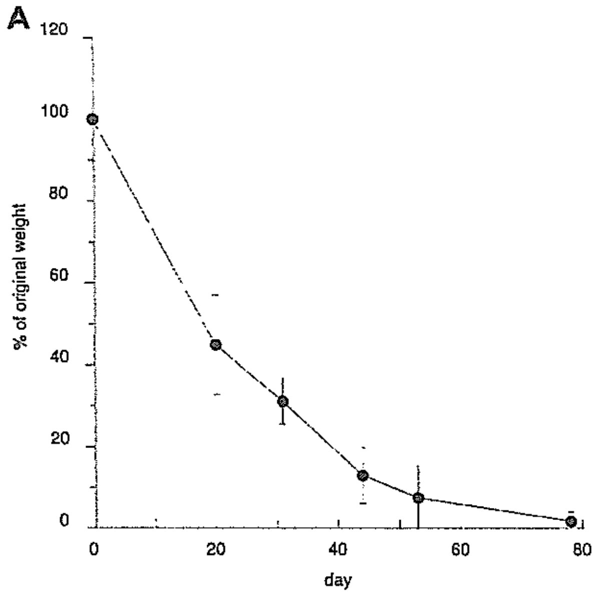


Figure 1. *Release of doxorubicin from the sheet in vitro. A) Spectrophotometric properties of doxorubicin. The light absorbance of doxorubicin was measured by continuous change in the wave length. Based on the figure, the absorbances at 232 nm and 480 nm were used for further determination of the drug 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



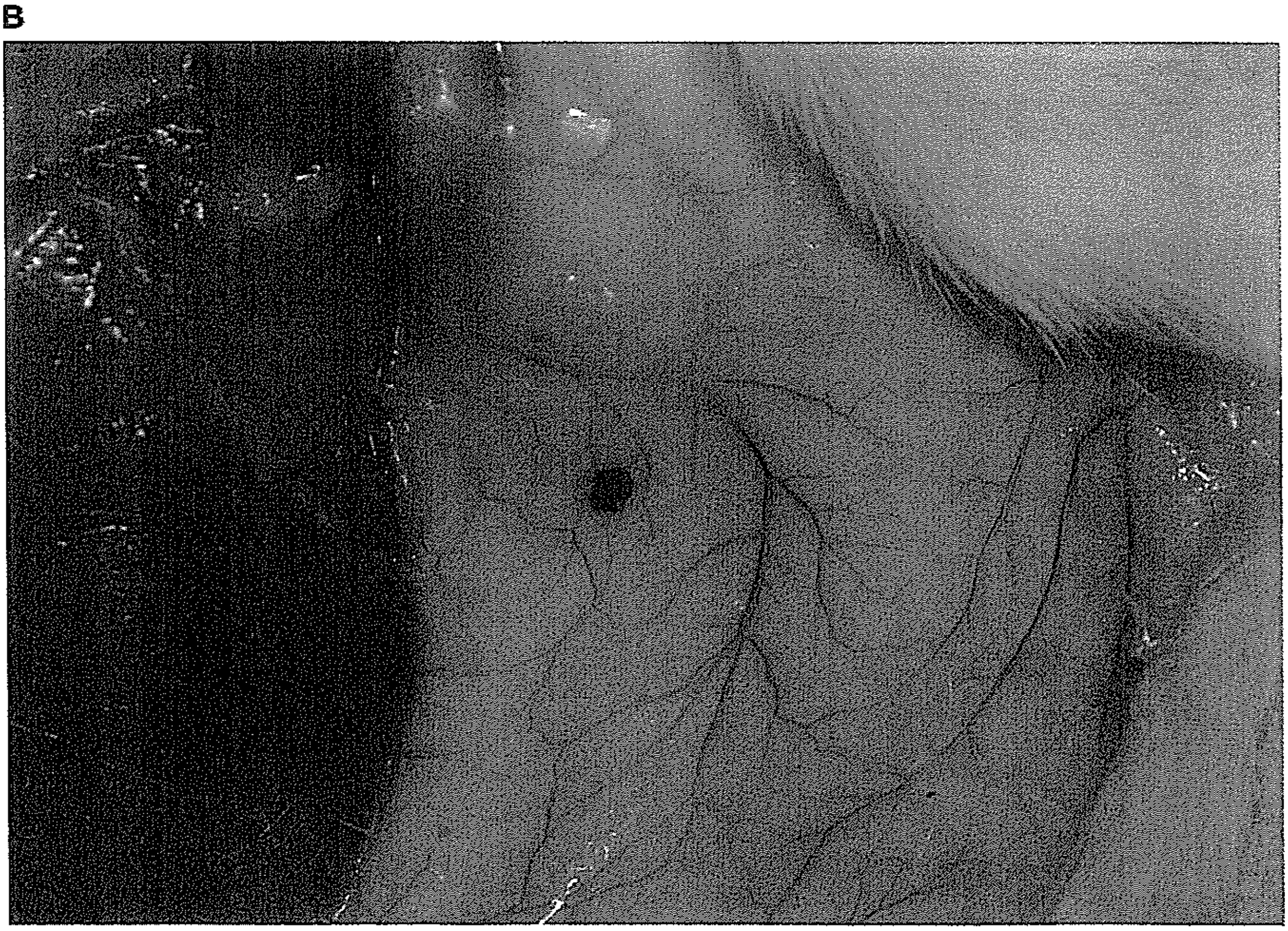
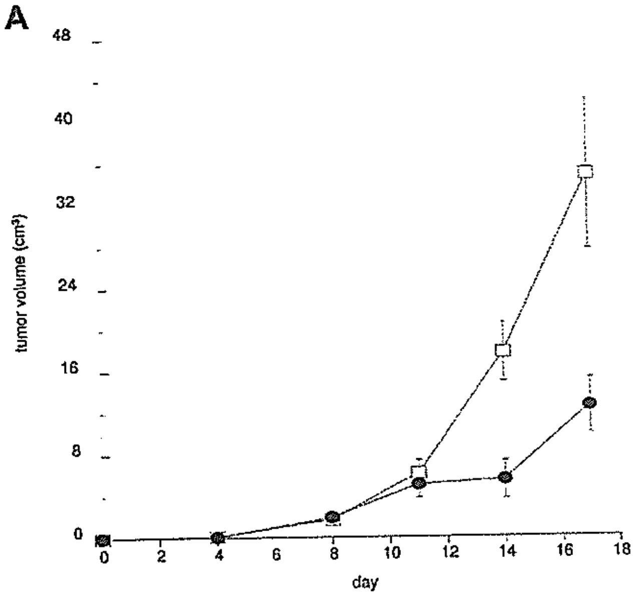


Figure 2. *Biodegradability of the sheet in vivo. A) The dry-weight of the implanted sheet was measured and biodegradability was expressed as a percentage of the original weight. The sheet degraded according to the passage of time. There was a rapid decrease in volume from the start of the experiment, 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

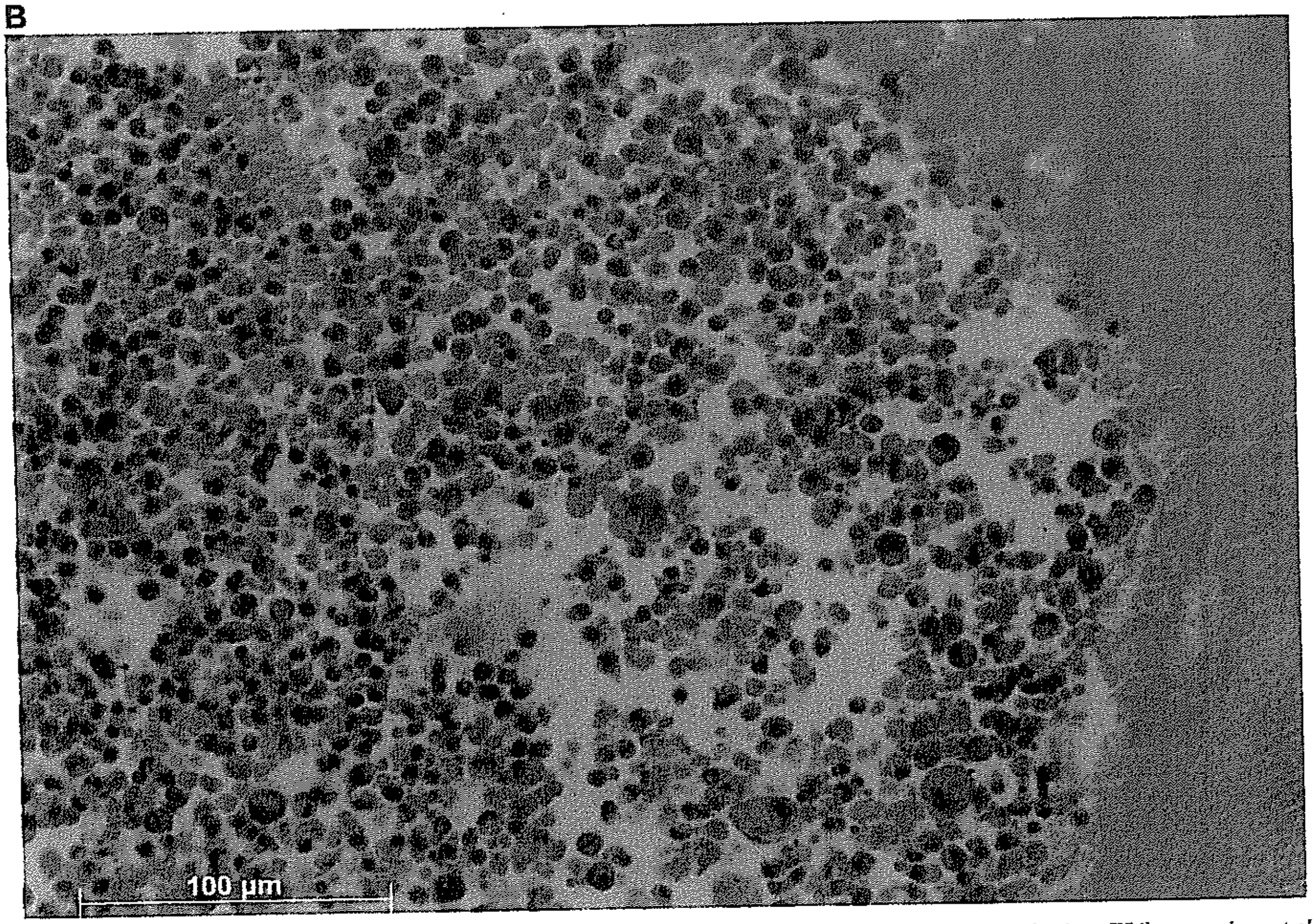


Figure 3. *Tumor growth inhibition by the sheet. A) After glioma cells were implanted, the tumor nodule was treated to the sheet. While tumors in control animals grew prosperously, treatment inhibited the expansion of the tumor. Mock sheet treatment (□); doxorubicin sheet treatment (●). There were differences 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.*

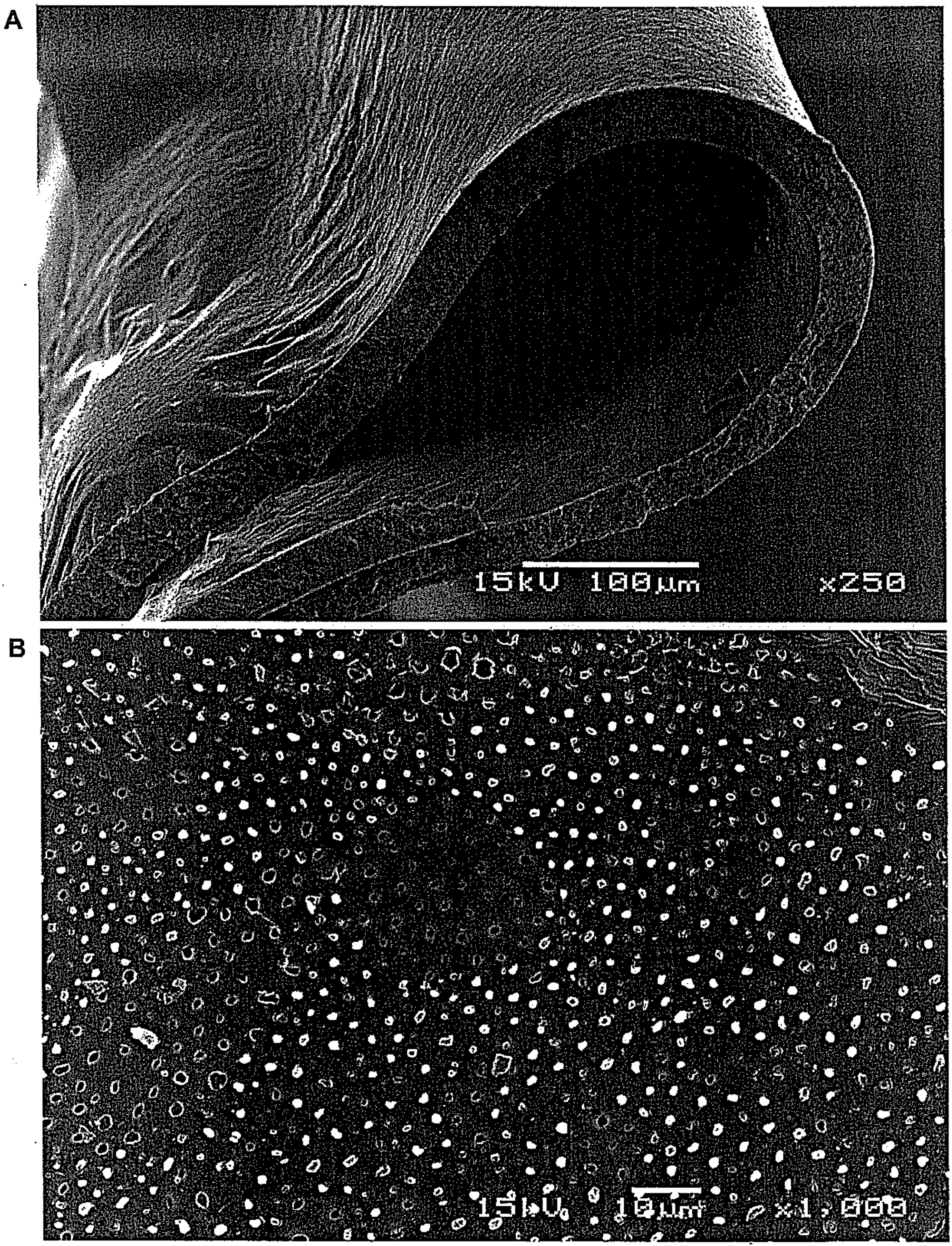


Figure 4. *Ultrastructure of doxorubicin sheet by electron microscopy. A-C) Pictures taken by scanning electron microscope, D) By transmission electron microscope. A) Overview: the sheet had a flexible texture with a thickness of 10 µm. B) Surface: the surface consisted of amorphous material with small 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.*

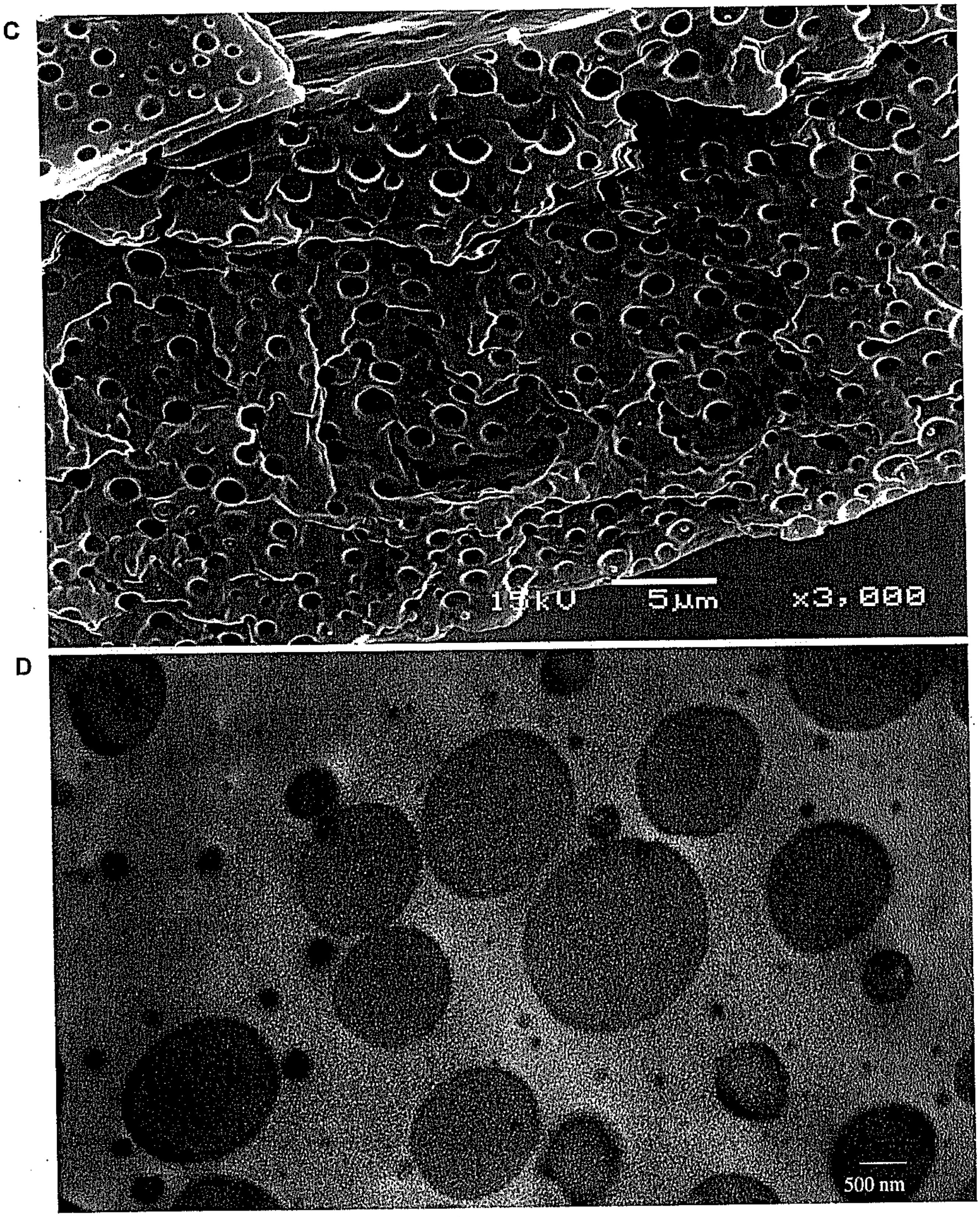


Figure 4. *continued*

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 administrated 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.

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Three-Way Bipolar Forceps: A Novel Bipolar Coagulator System for Nerve Stimulation and Detection of Nerve Potentials

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Abstract

Preoperative characterization of brain anatomy by magnetic resonance imaging and intraoperative functional characterization of the nervous system is essential in patients undergoing radical resection of brain tumors. A novel integrated system was developed combining conventional bipolar forceps with an electric stimulator and an oscilloscope. The system consists of a mechanical switching circuit allowing a wide range of electric characteristics and was designed to perform intraoperative electrophysiological studies, including functional mapping and measurements of motor evoked potentials (MEPs) and somatosensory evoked potentials (SEPs). This system achieved a significant reduction in exchange time (from 3.63 ± 1.00 sec to 1.12 ± 0.42 sec) between coagulation and stimulation, and reproducible measurement of MEPs from porcine limbs by cortical stimulation using the bipolar forceps. Functional mapping under awake craniotomy was carried out by cortical stimulation in patients with glioblastoma, and median nerve SEPs with high signal-to-noise ratio were elicited from the bipolar forceps on the sensory cortex of patients under general anesthesia. This integrated system is technically easy to operate and allows functional monitoring of an area that would otherwise be difficult to access using conventional methods. This three-way bipolar forceps system may reduce postoperative complications in patients undergoing neurosurgical procedures.

Key words: bipolar forceps, intraoperative monitoring, functional mapping, somatosensory evoked potential, motor evoked potential

Introduction

Functional monitoring of neurological activity using electrophysiological techniques is essential to reduce functional compromise during resection or coagulation of tumors involving the central nervous system. Such techniques include functional mapping by intraoperative electrical stimulation during awake surgery to identify the speech and motor areas,[10] motor evoked potential (MEP) monitoring under general anesthesia to assess efferent corticospinal motor tract integrity,[1] and somatosensory evoked potential (SEP) monitoring through the skull or brain surface to assess the activity of afferent peripheral nerves in the somatosensory tract.[9]

Electrophysiological techniques can establish neurological integrity, but are less useful for localizing eloquent areas within the surgical field that may be compromised during manipulation and result in postoperative functional impairments. Recently, techniques were described that allow the use of conventional bipolar forceps to function as stimulation electrodes.[6,11] However, this bipolar forceps is a dedicated probe for stimulation and must be exchanged for the coagulation forceps once the stimulation is complete.

Here we describe a novel surgical system (three-way bipolar forceps system) that integrates the functions of electrophysiological assessment (nerve stimulation and elicitation of potentials) with the conventional bipolar forceps used for resection and coagulation.

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