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International Journal of Pharmaceutics 213 (2001) 103–116

international journal of pharmaceutics

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Effect of cross-linking on the in vitro release kinetics of doxorubicin from gelatin implants

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Received 30 June 2000; received in revised form 24 October 2000; accepted 25 October 2000

Abstract

Doxorubicin is one of the most potent anti-tumor agents used generally in the treatment of bone cancer. Like other cancer chemotharepeutics, it produces undesirable side effects such as cardiotoxicity, which is especially severe when administrated via the conventional intravenous route. In order to minimize the systemic toxicities and to make this drug more suitable for the treatment of bone cancer, an implantable delivery system with cross-linked gelatin as the biodegradable matrix material was developed. This delivery system could possibly improve targeting of the drug as well as sustain the rate of release of the drug to the tumor. Glutaraldehyde was used as a cross-linking agent. Incorporation of glutaraldehyde in the matrix was needed to maintain the mechanical strength of the implant and to sustain the rate of release of the drug from the implant. Besides cross-linking the gelatin matrix, glutaraldehyde was found to cross-link the free amino group of doxorubicin. The effect of cross-linker concentration on the stability of the drug in the implant and on the rate and extent of release were also evaluated. In conclusion, cross-linked gelatin implants were developed for the local delivery of doxorubicin.

Keywords: Doxorubicin; Cross-linked gelatin; Implant; Glutaraldehyde

1. Introduction

Doxorubicin is an anthracycline cytostatic antibiotic with activity against a variety of malignancies, especially in the treatment of soft tissue, and bone sarcoma (Rosen et al., 1995; Coukell and Spencer, 1997). Like other cancer chemotherapeutics, doxorubicin is very toxic and has a narrow therapeutic index. The clinical usefulness of this drug is limited by its cumulative dose-related (Von Hoff et al., 1979; Pharmacia and Upjohn, 1998) and irreversible cardiotoxicity (Young et al., 1981; Keizer et al., 1990; Coukell and Spencer, 1997). In order to minimize the toxic side effects of the drug and enhance its therapeutic efficacy, various targeted drug delivery systems, such as liposomes (Hansen et al., 1995; Lasic, 1996; Gabizon and Martin, 1997), microspheres (Stolnik et al., 1995), polymeric micelles (Rolland et al., 1992; Kwon et al., 1994), and conjugates

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0378-5173/01/$ - see front matter
PII: S0378-5173(00)00651-7

(Dvorak et al., 1999), have been developed. Despite the improved therapeutic effectiveness of these targeted doxorubicin delivery systems for some solid tumors (Coukell and Spencer, 1997; Muggia et al., 1997; Minko et al., 2000), their application in the treatment of bone sarcomas is limited. The major problem associated with the treatment of bone sarcomas by systemic chemotherapy is the difficulty of achieving therapeutic concentration of drugs at the tumor sites without affecting the surrounding tissues. Due to the cardiotoxicity and the narrow therapeutic index of doxorubicin, substantial increase in systemic dose to achieve high concentration of the drug at the target site is not possible. Moreover, treatment with doxorubicin is usually discontinued after patients have received a lifetime dose of 400–500 mg/m^2 (McEvoy et al., 2000). Thirty to 40% of patients experience cardiotoxicity after receiving a cumulative dose of 700 mg/m^2 (Sadee and Torti, 1987). Secondly, since bones in general are moderately perfused organs, the concentration of systemically administered drug at the bony cancerous site is likely to be very low. It has been reported that administration of a 30 mg/m^2 of doxorubicin as an intravenous bolus dose only resulted in a marrow drug concentration of 0.52 μg/g, 2.5 h after administration (Cohen and Chan, 1981). Most of the available doxorubicin delivery systems, such as liposomes (pegylated and conventional), polymeric micelles, or microspheres, are usually administered intravenously. Thus, currently available dosage forms and drug delivery systems used for doxorubicin are not adequate for the treatment of bone sarcomas. In summary, the problems associated with the intravenous administration route of doxorubicin for the treatment of bone cancer are: (i) systemic cardiac toxicity caused by the high levels of doxorubicin (Young et al., 1975); (ii) the drug concentration at a cancerous site is likely to be low because bone is a moderately perfused organ; and (iii) the narrow therapeutic index of doxorubicin does not permit substantial increases in the dose administrated. Therefore, a localized and targeted drug delivery system for an antitumor agent is a logical choice and effective means of minimizing the problems that may occur during the conventional administration of antineoplastic drug for the treatment of bone sarcomas.

Hydroxyapatite implants containing doxorubicin have been developed and tested successfully by Itokazu et al. (1996) in an in vivo model for the treatment of osteogenic sarcoma. According to this study, an implant containing 0.3 mg of doxorubicin was placed in mice, and the concentration of the drug was determined during a 4-week period. The drug concentration after 4 weeks in the implant site, plasma, kidney, and liver was found to be 12.3, less than 3, 0.019 and 0.026 μg/g, respectively. Results of this study clearly indicate that the use of a local delivery system reduces the dose to be administered as well as the risk of systemic toxicity compared to the conventional systemic administration.

The aim of this study was to develop an implantable and biodegradable drug delivery system containing doxorubicin for the treatment of bone cancer. We evaluated the usage of cross-linked gelatin as a biodegradable matrix material in the design of a drug delivery system for doxorubicin. Since both gelatin and doxorubicin are hydrophilic in nature, one can expect that the rate of release of doxorubicin from a non-cross-linked gelatin implant will be very rapid and complete. Therefore, a suitable cross-linking agent (glutaraldehyde) was identified and utilized in our laboratory to control the release of doxorubicin from the gelatin matrix as well as to maintain the mechanical strength of the implants. In this study we report the cross-linking effect of various concentrations of glutaraldehyde in gelatin implants and its overall effect on the rate of release of doxorubicin. A possible cross-linking of glutaraldehyde with the free amino group of doxorubicin has also been identified.

2. Materials and methods

2.1. Materials

Doxorubicin hydrochloride, Trizma® (Tris[hydroxymethyl]aminomethane) base, Trizmao® hydrochloride were obtained from Sigma (St. Louis, MO). Daunomycin hydrochloride was obtained

from Fluka (Milwaukee, WI). Acetonitrile, water (HPLC grade), glacial acetic acid, glutaraldehyde solution (50%, w/w), glutaraldehyde sodium bisulfite addition compound (molecular weight 308.23) (reagent grade) were all obtained from Fisher Scientific (Pittsburgh, PA). Absorbable gelatin powder (Gelfoam®) was kindly provided by Pharmacia and Upjohn Company (Kalamazoo, MI).

2.2. Analytical method used for the determination of doxorubicin

2.2.1. Chromatography

The HPLC system consisted of the solvent delivery module LC-10AT (Shimadzu Corporation, Kyoto, Japan), a manual injector (20 μl loop, Rheodyne, Cotati, CA), a Waters 470-fluorescent detector, and a Waters 745 data module (Waters Chromatography Division, Milfold, MA). The separation was carried out on a 50 × 1 mm i.d. (5 μm) reversed-phase C18 Luna column (Phenomenex, Torrance, CA). The mobile phase consisted of water:acetonitrile:acetic acid (77:22:1, v/v/v) with an apparent pH of 2.8. The flow rate was maintained at 0.1 ml/min. Daunomycin hydrochloride was used as the internal standard. The effluents were monitored at $\lambda_{Ex} = 505$ nm and $\lambda_{Em} = 550$ nm. All chromatographic analyses were performed at room temperature.

2.2.2. Standard solutions

Doxorubicin (0.005–0.05 μg/ml) and daunomycin (0.08 μg/ml) were prepared in the mobile phase. Doxorubicin (0.14 mg) was dissolved in 100 ml mobile phase in a volumetric flask. Various standard solutions were then prepared from this stock solution after adequate dilution with mobile phase. Daunomycin (0.13 mg) was dissolved in 100 ml mobile phase to make the stock solution. The standard solution of daunomycin was prepared by diluting 3.0 ml of the stock solution in a 50-ml volumetric flask with the mobile phase.

2.2.3. Sample preparation

To 900 μl of doxorubicin standard solutions, or the solution (unknown) to be analyzed for the drug content, 100 μl of internal standard solution was added and vortexed for 10 s. This mixture (20 μl) was injected directly into the HPLC.

2.2.4. Quantitation

The ratios of the peak height of doxorubicin to that of the internal standard were calculated. The unknown doxorubicin concentration was determined from the regression equation relating the peak-height ratios (PHR) of the standards to their nominal concentration (Zhao and Dash, 1999).

2.3. Formulation of the implants

2.3.1. Non-cross-linked implants

One-part gelatin (Gelfoam®), two-part water, and doxorubicin were mixed in a glass beaker and forced through a 1-ml plastic syringe to form the cylindrical implant (12 mm long, 3.5 mm diameter), which was then cut into appropriate sizes to yield implants of definite dimensions (4 mm long, 3.5mm diameter). The implants were kept in a desiccator for 2–6 days prior to the in vitro release studies. The drug load was kept around 0.1% (w/w).

2.3.2. Implants with cross-linking agents

Glutaraldehyde sodium bisulfite addition compound: 20% (w/w) glutaraldehyde sodium bisulfite addition compound in aqueous solution was prepared by dissolving 10 g of such compound in 50 ml water in a volumetric flask. To use this as a cross-linking agent, 5, 10 and 15% (v/v) of water was substituted, respectively, by equal volume of this 20% (w/w) addition compound aqueous solution when the implants were prepared, the rest of the procedures were exactly the same as described above. The theoretical drug load was kept around 0. 1% (w/w).

Glutaraldehyde solution (50%, w/w): to use this solution as a cross-linking agent, 0.5, 1, 2 and 5% (v/v) of water was substituted, respectively, by an equal volume of glutaraldehyde solution (50%, w/w). The theoretical drug load was kept at around 0.1% (w/w). One more batch of an implant was also fabricated with a 5% (v/v) cross-linking, agent but with a higher theoretical drug load (1.2% w/w).

2.4. Stability of doxorubicin in solution

The stability of doxorubicin in the release medium was carried out in a closed 50 ml siliconized glass flask, which was kept at 37°C in a Precision reciprocal shaking water bath (Precision Scientific, Winchester, VA), shaken at 80 rpm. The doxorubicin content was determined by HPLC immediately after sample collection and also after keeping the samples at 4°C for 4 days.

The effect of glutaraldehyde concentration on the stability of doxorubicin in solutions was carried out over a period of 4 days at 37°C. Instead of the implant, the mixture of 16 μl doxorubicin aqueous solution (1.97 μg/ml) and 5 μl glutaraldehyde (50%, w/w) were kept in a 40 ml release medium. For the control samples, glutaraldehyde was substituted by water. The rest of the procedures were exactly the same as those used for in vitro release study.

2.5. Determination of the drug load in the implant

A weighed doxorubicin implant was soaked in 2 ml mobile phase at 4°C overnight, and then crushed by a porcelain mortar and pestle and diluted to 50 ml with mobile phase in a volumetric flask. The sample was sonicated for 25 min and filtered through a 0.45 μm syringe filter. The doxorubicin content in the filtrate was then determined by HPLC. For every batch of implants, the drug loading studies were carried out in triplicate.

2.6. In vitro release study

The in vitro release of doxorubicin from the implants was carried out in a closed 50 ml polypropylene flask. The weighed implants were kept in 40 ml of 0.05 N Tris buffer (pH 7.4). The flasks were shaken at 37°C by a Precision reciprocal shaking water bath at 80 rpm. One milliter of the release medium was collected at corresponding time intervals (0.33, 0.67, 1, 2, 6, 12, 24, 36, 48, 60, 72, 96, 120, 144, 168 h) and replaced with 1 ml of fresh buffer. In vitro release studies were carried out in triplicate. The doxorubicin content in the release medium was determined by HPLC.

2.7. Effect of cross-linking on the in vitro drug release

The effect of cross-linking if any, between doxorubicin and glutaraldehyde on the release of doxorubicin was conducted under similar conditions used for the in vitro release study. A doxorubicin aqueous solution (0.98 μg/ml, 125 μl) was mixed with glutaraldehyde (50%, w/w, 20 μl). For the control samples, glutaraldehyde was substituted by equal volume of water. The mixtures were kept in a desiccator until complete drying for 3 days prior to the release study.

2.8. Scanning electron microscopic (SEM) studies

The morphology of the samples was evaluated by a Philips XL20 scanning electron microscopy. The voltage was set at 1 kV. No coatings were applied to those samples. The scanning electron (SE) mode was selected to examine the overall surface morphology. Through lens detection (TLD) mode was selected to view the detailed surface structures of the samples. Back scattered (BS) mode was selected to distinguish the phases containing various chemical compositions.

3. Results and discussion

3.1. Stability of doxorubicin under release conditions

Doxorubicin hydrochloride is very stable in the solid state. It has been stored for years at room temperature without any loss in potency or indications of degradation (Vigevani and Williamson, 1980). However, the stability of Doxorubicin in aqueous solution can be affected by many factors, such as pH, buffer concentrations, temperature (Janssen et al., 1985), light, and metal ions (Beijnen et al., 1986). Doxorubicin is stable in acidic solutions in the pH range 3.0–6.5, but rapid decomposition occurs at a higher pH (6.5–12) (Vigevani and Williamson, 1980; Beijnen et al., 1986). The pH of the release medium in this study was kept at 7.4.

The degradation profiles of doxorubicin in the release medium at 37°C are shown in Fig. 1. Approximately 30–40% (w/w) of the drug was degraded within 48 h at this temperature. Approximately 10% or more doxorubicin was degraded when these samples were kept at 4°C for 4 additional days after collection. In order to account for this degradation, a control was run each time during the in vitro release studies.

Doxorubicin has also been found to adsorb onto various materials such as polytetrafluoroethylene (PTFE), glass, polyethylene, and 'plastic' containers (Hahn, 1978; Tomlinson and Malspeis, 1982), but not to siliconized glass or polypropylene (Tomlinson and Malspeis, 1982; Bosanquet, 1986). Therefore, polypropylene flasks were used in the release studies and siliconized polypropylene tubes were used for storing samples.

3.2. In vitro release of doxorubicin from gelatin implants

Gelfoam is an absorbable sterile gelatin powder commercially available for application to bleeding surfaces. Gelfoam has also been used extensively as a carrier in ocular devices for the controlled release of many drugs, such as phenylephrine and tropicamide (Lee et al., 1999), insulin (Lee et al., 1997), and pilocarpine (Simamora et al., 1998). Gelatin, a macromolecular protein derived from collagen, is a non-toxic and biodegradable material widely used in food and drug industry. It is composed of 18 different types of amino acids linked together to form a branched chainlike structure with free amino-guanidine basic and carboxyl acidic groups (Deasy, 1984), which contributes to the high hydrophilic characteristic of gelatin. Gelatin alone or in conjunction with other materials has been utilized in the fabrication of various drug delivery systems (Park and Kim, 1997; Li et al., 1998; Chowdhury and Mitra, 1999; Cortesi et al., 1999). Leo et al. (1999), have reported the release of doxorubicin from doxorubicin-gelatin nanoparticle conjugates. Leo et al. have also identified the possible interaction of free amino group of doxorubicin and glutaraldehyde in these nanoparticle conjugates (Leo et al., 1997).

In this study, gelatin was used as the matrix material for fabrication of the implant. The in vitro release of doxorubicin from non-cross-linked gelatin implants is depicted in Fig. 2. As expected, 80–82% (w/w) of this hydrophilic drug was released from such an implant within 15 h. The rapid decline in the concentration of the drug in the release medium indicated a rapid degradation of the free drug at 37°C in solution. The hydrophilic nature of gelatin resulted in the rapid penetration of the release medium into the matrix; and consequently, the rapid diffusion of the drug from such an implants. More importantly, these implants had poor mechanical strength for implantation. Therefore, the non-cross-linked gelatin implant was found unsuitable for the design of an implantable delivery system for doxorubicin for this investigation.

In order to overcome the above difficulties, cross-linking agents were introduced into these implants. Glutaraldehyde, a less toxic but more effective dehydrating agent, was evaluated. It is commonly used to harden the gelatin wall by cross-linking the amino group of protein (Richards and Knowles, 1968; Takenaka et al., 1979; Deasy, 1984). Glutaraldehyde is available commercially as: (a) a sodium bisulfite addition compound; and (b) an aqueous solution (50% w/w).

The in vitro release of doxorubicin from gelatin implants using sodium bisulfite addition compound as the cross-linker was investigated. An increase in the cross-linker concentration, did not affect the release kinetics of the drug. The mechanical strength of such cross-linked implants was also not improved much when compared with that of the non-cross-linked ones. Result of this study clearly suggests that the sodium bisulfite addition compound is not acting as a cross-linker for gelatin under the experimental conditions described here.

The release profiles of doxorubicin from the gelatin implants, which were cross-linked by various concentrations of aqueous glutaraldehyde solution, are shown in Fig. 3. As expected, increase in the glutaraldehyde concentration decreased the rate of release of the drug from these implants. The differences in the rate of release of the drug

from theses implants can be explained by the fact that cross-linking process usually hardens the gelatin matrix and also provides increased resistance for the penetration of release medium into the implant; Therefore, the diffusivity of the drug through the matrix material is lowered (Domb et al., 1990; Warren and Kellaway, 1998). We were also interested in evaluating a possible mechanism of doxorubicin release from these implants. A linear relationship was observed when the fraction of drug released at the initial phase of the release studies (12–72 h) was plotted against the square root of time. The results of this study were summarized in Table 1, which suggest that the release of doxorubicin from these formulations occurred by a matrix diffusion controlled mechanism as described by Higuchi (1961). The slope of the lines which is a measure of the rate of doxorubicin release from each of the delivery systems was found to be different as depicted in column 4. Interestingly, the rate of doxorubicin release from the implants with a higher drug load was found to be slower as compared with a lower drug load implant (last two rows of Table 1). In both these implants, the amount of cross-linker was kept identical. A decrease in the rate of release of doxorubicin from an implant containing a higher doxorubicin load can be explained if the drug cross-links with the glutaraldehyde during the preparation of the implant. Therefore, under such circumstances, the amount of free drug in the implant will be lower as compared to a non-cross-linked implant.

The free drug concentration in the implants was then determined by the LC method and results are shown in Fig. 4. The free drug concentration was greatly decreased when the concentration of the cross-linker was increased in the implant. The drug loading determined in this study was actually the free doxorubicin concentration in the implants, since only the free drug could be determined by the analytical method used in this study. This decrease in drug load can be explained either by degradation of doxorubicin in presence of glutaraldehyde or by self-cross-linking between doxorubicin and glutaraldehyde. The stability of doxorubicin in presence of glutaraldehyde in solution was evaluated and shown in Fig. 5. In this study, the amount of doxorubicin used reflected the theoretical amount of the drug present in a typical implant. The concentration of glutaraldehyde used was identical to an implant containing

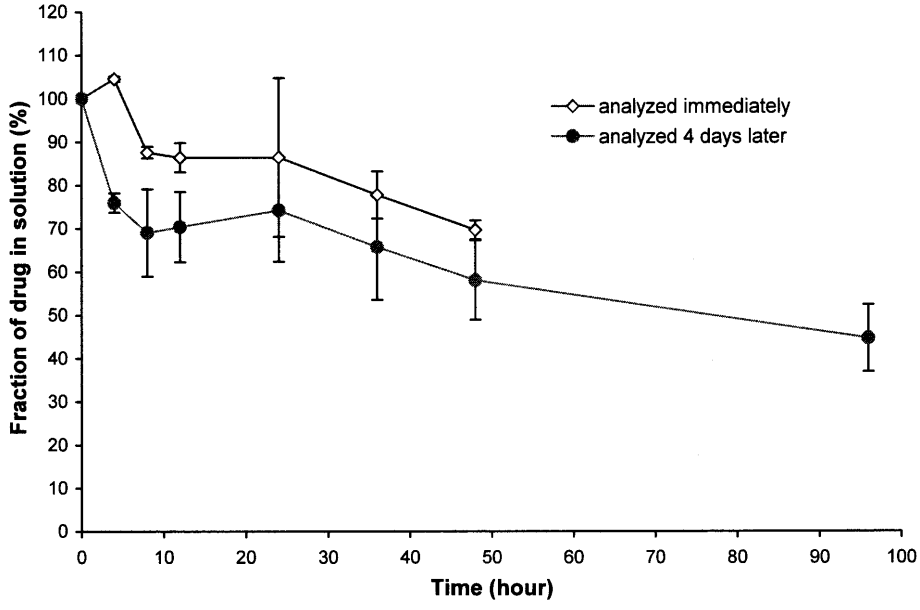

Fig. 1. The stability of doxorubicin in the release medium at 37°C. Values expressed as mean ± S.D. ($n = 3$).

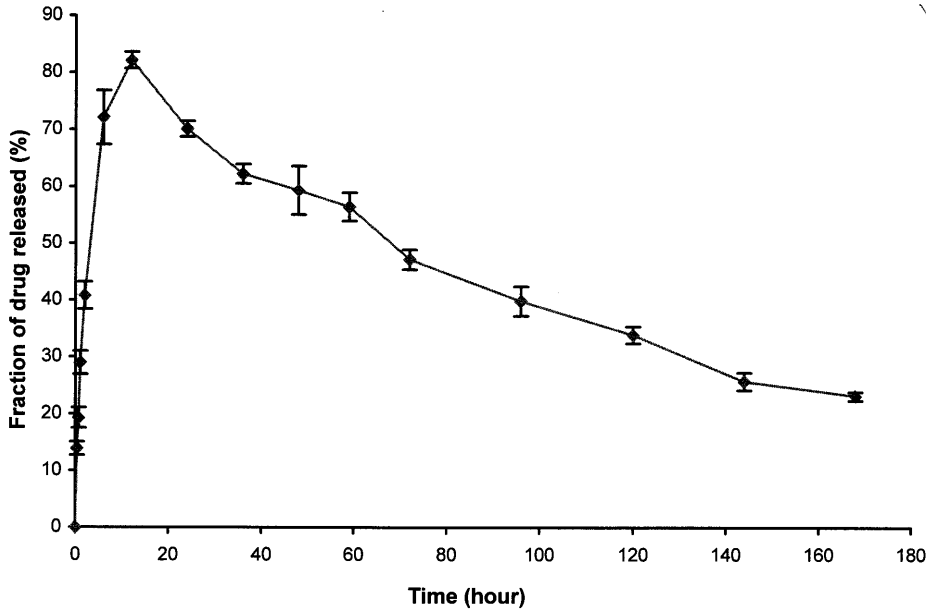

Fig. 2. The in vitro release profiles of doxorubicin from non-cross-linked gelatin implants. Values expressed as mean ± S.D. ($n = 3$). The actual drug load in the implant determined by LC method was 0.1085% (w/w).

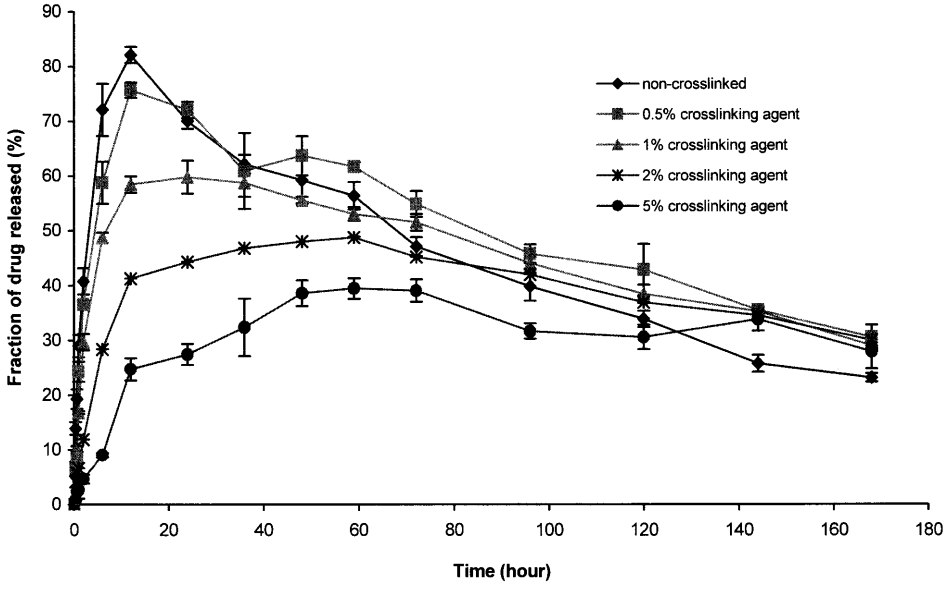

Fig. 3. The in vitro release profiles of doxorubicin from gelatin implants cross-linked by glutaraldehyde aqueous solution (50%, w/w). The experimental drug load for the implants containing 0% (non-cross-linked), 0.5, 1, 2 and 5% (v/v) of cross-linking agents were 0.109, 0.070, 0.054, 0.027 and 0.010% (w/w), respectively. Values expressed as mean ± S.D. ($n = 3$).

5% (v/v) of the cross-linking agent. As shown in Fig. 5, there was no significant difference in the degradation pattern in both cases. This result indicated that the presence of glutaraldehyde did not affect the stability of the drug in solution. Therefore, the decrease in the drug load in the

implant containing glutaraldehyde was believed to be due to the self-cross-linking between the drug and the cross-linking agent. Our findings were also in good agreement with the results already reported by Leo et al., (1997).

The effect of the cross-linking on the in vitro release of doxorubicin, from the dried physical mixture is shown in Fig. 6. In this study, the amount of doxorubicin, and cross-linking agent used were identical to an implant tested in an in vitro study. The rate and extent of drug release from the dried mixture containing glutaraldehyde was found to be substantially lower as compared to the control sample. Therefore, we hypothesized that the drug might be cross-linked with glutaraldehyde when the mixture was dried. In the cross-linking mechanism of protein with glutaraldehyde the amino groups of protein react with the aldehyde groups (Deasy, 1984; Richards and Knowles, 1968). Since doxorubicin also has a free amino group in its glucose portion of the molecule, we postulate that the drug might also be cross-linked in presence of glutaraldehyde as well. In order to test our hypothesis, we prepared implants with 5% (v/v) of glutaraldehyde and with a very high doxorubicin loading (12 times more

Table 1
Comparison of the slope, Y-intercept and R^2 values of the square root of time profiles[a]

Implants with various weight fractions of cross-linking agents	Time span (in hours) of the release data used to plot the square root of time profiles	Value of the Y-intercept	Slope	R^2
Non-cross-linked	12	1.705	25.23	0.972
0.5%	12	−2.844	23.65	0.966
1%	12	−0.935	18.29	0.972
2%	12	−3.435	12.56	0.980
5%	72	−1.051	5.35	0.963
5% with high drug load	72	−2.554	3.42	0.985

[a] The initial portion of the release data from Fig. 3 (as indicated in column 2) were plotted against the square root of time for determining these parameters.

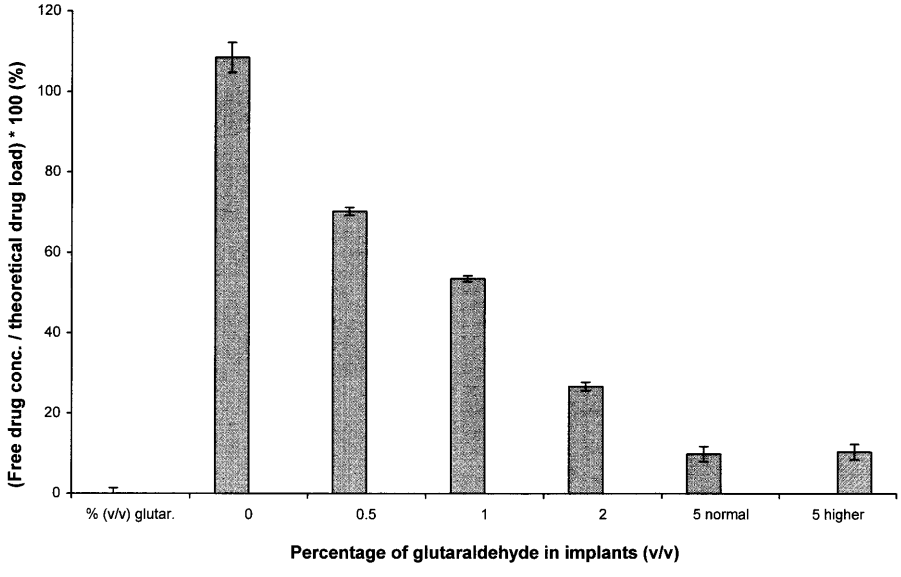

Fig. 4. Relationship of free drug concentration vs. the percentage (v/v) of glutaraldehyde solution (50%, w/w) in the implants. Values expressed as mean ± S.D. ($n = 3$).

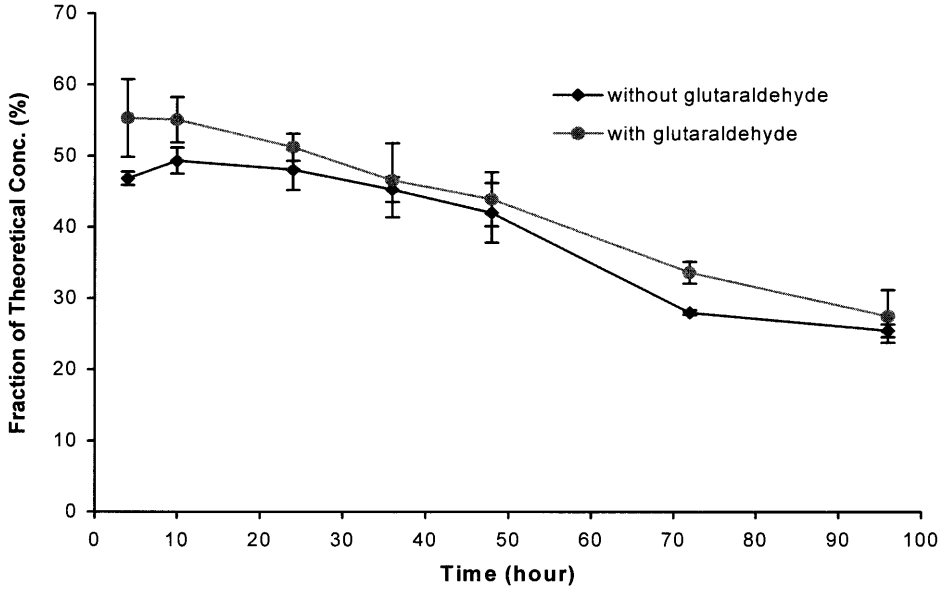

Fig. 5. The effect of glutaraldehyde solution (50%, w/w) on the stability of doxorubicin in the release medium at 37°C. The concentration of doxorubicin in both the solutions was identical. The glutaraldehyde concentration used was identical to an implant containing 5% (v/v) of cross-linking agent. Values expressed as mean ± S.D. ($n = 3$).

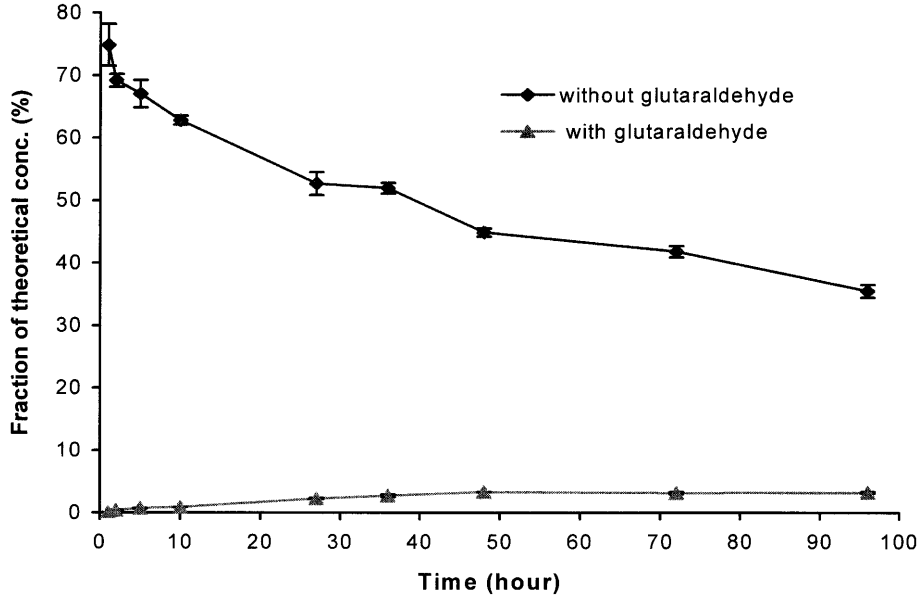

Fig. 6. The effect of cross-linking between doxorubicin and glutaraldehyde on the release of free drug from the dried physical mixture. The concentration of doxorubicin in both the dried mixtures was identical. The glutaraldehyde concentration used was identical to an implant containing 5% (v/v) of cross-linking agent. Values expressed as mean ± S.D. ($n = 3$).

than the usual drug load of 0.1%, w/w). Interestingly, the free drug load in such implants was very low (close to the theoretical normal drug load, 0.1%, w/w). This result of the proportional decrease of the free drug load in the 5% (v/v) cross-linked implants partly supported our hy-

pothesis that the drug might cross-link with the cross-linking agent as well in the implant. Fig. 7 depicts the in vitro release profiles of the drug from such implants. Even though the free drug loading (determined by HPLC) in both types of implants were the same, the release rate of the drug from the cross-linked implants was substantially lower. This may be due to the cross-linking effect between glutaraldehyde and gelatin, which is considered to increase the resistance of the matrix material for the diffusion of the drug through the hardened matrix and consequently retard the release rate of the drug from such implants (Domb et al., 1990; Warren and Kellaway, 1998).

3.3. Effect of long term storage on the stability of the drug in the implant

The stability of doxorubicin in the implants on long-term storage is summarized in Table 2. The implants were stored in a desiccator at room temperature up to 5 months. The drug load in the implant at time zero and after 5 months of storage was determined. No significant degradation of doxorubicin was noticed within 5 months at a very low cross-linker concentration or without any cross-linker. However, at a higher cross-linker concentration, degradation of doxorubicin was noticed in the matrix. This degradation was possibly induced by higher concentration of glutaraldehyde which might help in cross-linking of the drug itself, therefore, less free drug was available and detected by the assay procedure.

3.4. Effect of cross-linking on the microstructure of the implants

The morphology of the gelatin implants before and after in vitro release studies were evaluated using SEM. Fig. 8(a) and (b) represent the micrographs of gelatin implants without cross-linker before and after release studies, respectively. Fig. 8(c) and (d) represents the scanning electron micrographs of cross-linked implants with 5% (w/w)

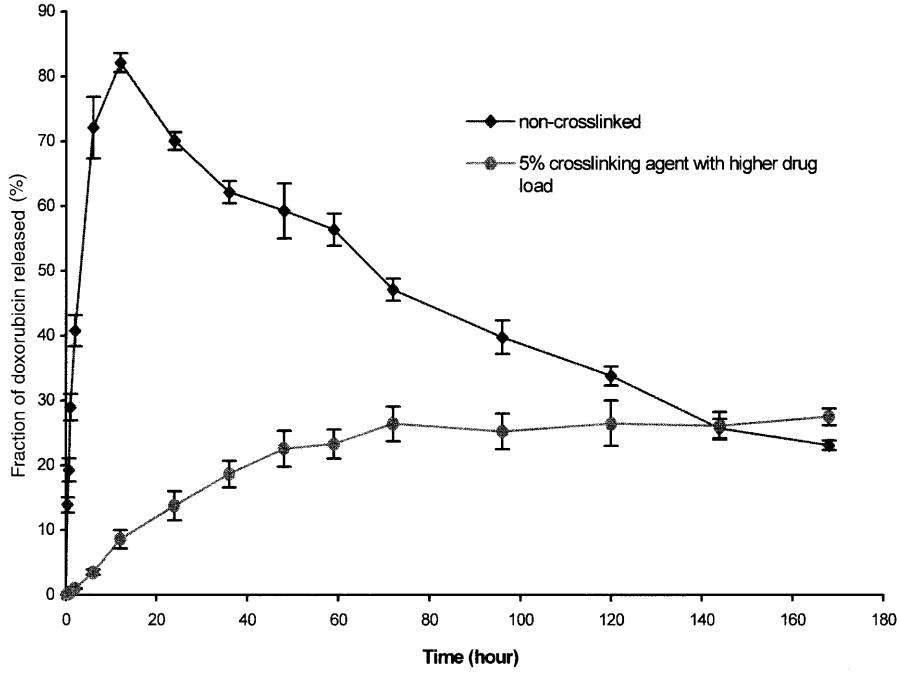

Fig. 7. The release profile of doxorubicin from 5% (v/v), glutaraldehyde solution) cross-linked implants with the same free drug concentration as the non-cross-linked control. The experimental drug loading (determined by HPLC) for non-cross-linked and cross-linked implants were 0.1085 and 0.1249% (w/w), respectively. Values expressed as mean ± S.D. ($n = 3$).

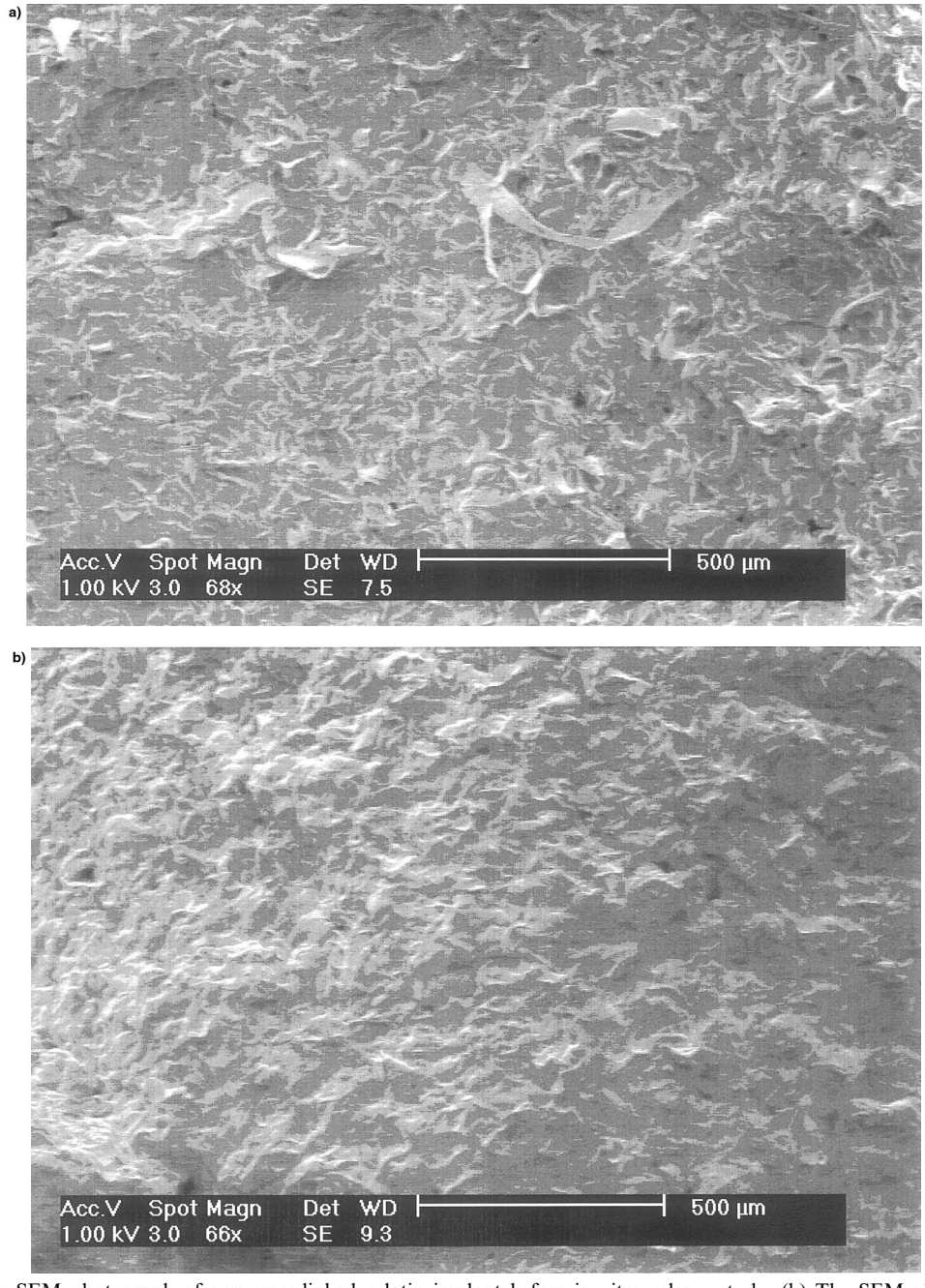

Fig. 8. (a) The SEM photograph of non-cross-linked gelatin implant before in vitro release study. (b) The SEM photograph of non-cross-linked gelatin implant after 7 days of in vitro release study. (c) The SEM photograph of 5% (v/v) glutaraldehyde cross-linked gelatin implant before in vitro release study. (d) The SEM photograph of 5% (v/v) glutaraldehyde cross-linked gelatin implant after 7 days of in vitro release study.

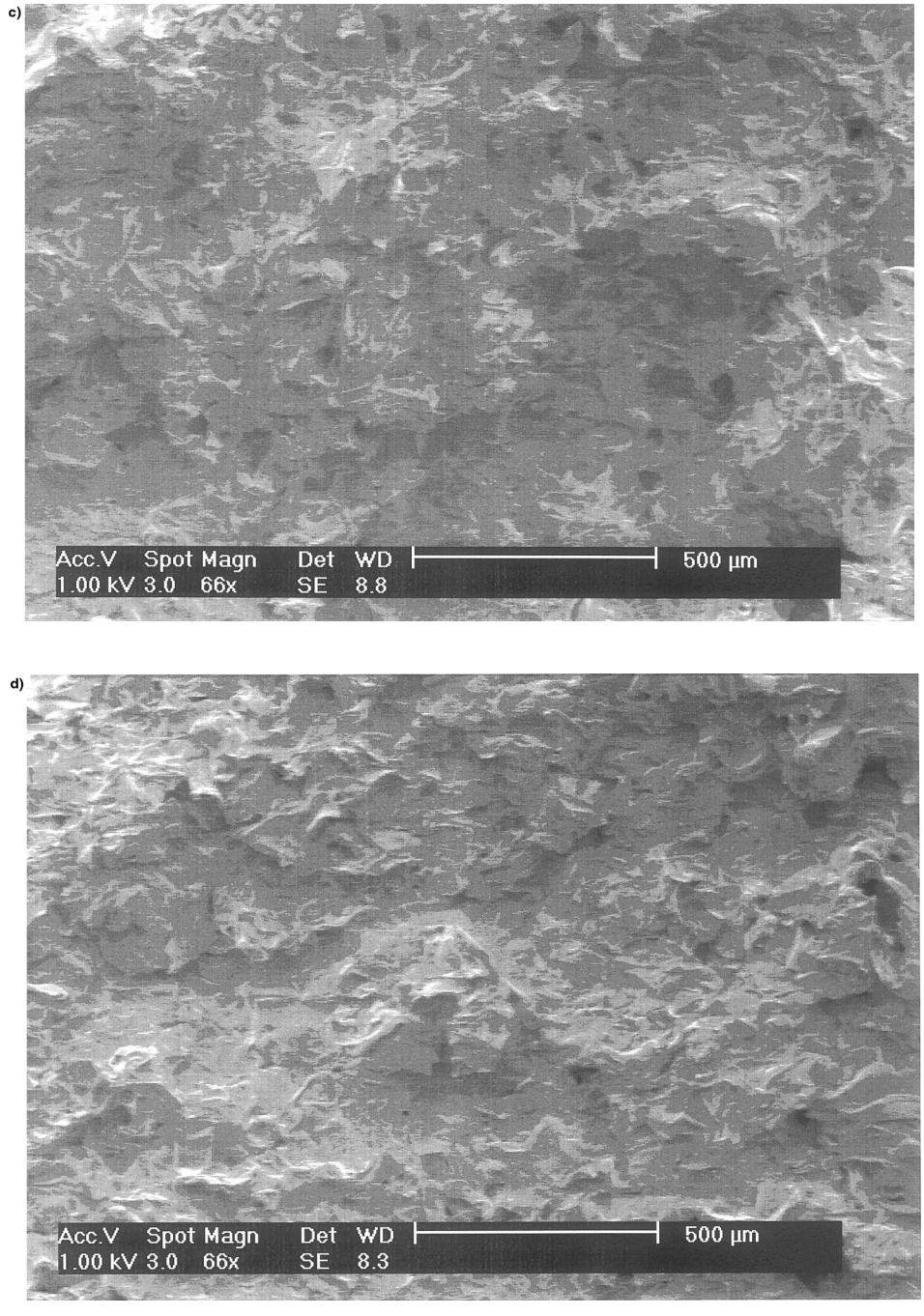

Fig. 8. (*Continued*)

Table 2
Effect of long-term storage (up to 5 months) on free drug loading of the gelatin implants

Implant type	Glutaraldehyde solution (% v/v)	Drug load at time zero (% w/w)	Drug load after 5 months (% w/w)	Decrease in drug load (%)
Non-cross-linked	0.0	0.109 ± 0.001*	0.109 ± 0.050*	0.00
Cross-linked	0.5	0.070 ± 0.004	0.070 ± 0.015	0.00
Cross-linked	1.0	0.054 ± 0.001	0.049 ± 0.001	9.26
Cross-linked	2.0	0.027 ± 0.001	0.023 ± 0.002	14.81
Cross-linked	5.0	0.010 ± 0.011	0.008 ± 0.000	20.00
Cross-linked	5.0 with higher drug load	0.125 ± 0.019	0.103 ± 0.003	17.60

* Mean $\pm$ S.D.; $n = 3$.

glutaraldehyde before and after release studies respectively. The SEM photographs clearly show a difference in the microstructure, which is contributed by the swelling of the matrix during the in vitro release studies. Comparison of the micrographs of the non-cross-linked and cross-linked implants revealed that addition of glutaraldehyde substantially changed the microstructure of the implants due to the cross-linking. This change in microstructure is partly responsible for the change in the rate and extent of drug released from such implants.

4. Conclusions

An implantable delivery system for doxorubicin with biodegradable matrix material (gelatin) was developed. Glutaraldehyde can be used as a cross-linking agent in this system in order to maintain mechanical strength of the implant and to sustain the rate of release of the drug from the implant. Glutaraldehyde also reduces the free doxorubicin concentration in the implants probably due to the cross-linking with the drug. The above implantable delivery system for doxorubicin with non-toxic and biodegradable matrix material (cross-linked gelatin) may offer better promise for its use in the clinical treatment of bone sarcoma.

Acknowledgements

The authors would like to thank Pharmacia & Upjohn Company for the generous gift of Gelfoam® samples.

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