Development of a Polymeric Surgical Paste Formulation for Taxol

Charles I. Winternitz,[1] John K. Jackson,[1] Ann Marie Oktaba,[2] and Helen M. Burt[1,3]

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Purpose. To develop and characterize a biodegradable polymeric sustained release surgical paste formulation for taxol.

Methods. Taxol was incorporated into poly(ϵ-caprolactone) (PCL) or blends of PCL with methoxypolyethylene glycol, MW 350 (MePEG). The surgical pastes were characterized using gel permeation chromatography, thermal analysis, scanning electron microscopy, and a tensile strength tester. In vitro release data for taxol from the surgical paste formulations was carried out at 37°C in phosphate buffered saline, pH 7.4, using an HPLC assay for taxol. Antiangiogenic activity of the formulations were assessed using a chick chorioallantoic membrane assay (CAM).

Results. The addition of up to 30% MePEG in PCL decreased the melting point of PCL by 5°C and the tensile strength by 152.7 N/cm^2 to 26.7 N/cm^2 but increased the degree of PCL crystallinity from 42% to 51%. Taxol showed a biphasic in vitro release profile composed of a burst phase lasting 1 or 2 days followed by a period of slow sustained drug release. There was no significant difference in the release profiles of taxol from two different sources of PCL. The addition of MePEG increased the amount of water taken up by the polymer blends but decreased the rate of taxol release. The formulations were shown to have antiangiogenic activity by the CAM assay at levels as low as 0.1% taxol using 3 mg surgical paste pellets.

Conclusions. Our surgical paste formulations for taxol give sustained release while having physical properties which can be adjusted using additives.

KEY WORDS: taxol; poly(ϵ-caprolactone); methoxypolyethylene glycol; biodegradable polymers; sustained release.

INTRODUCTION

Taxol has shown promise as an effective anticancer agent (1). Its current formulation, a 50:50 mix of Cremophor EL®, and dehydrated alcohol USP, has been associated with a high incidence of hypersensitivity in patients in addition to the other adverse effects of taxol including neutropenia, peripheral neuropathy, arthralgia, myalgia, mucositis, nausea and vomiting, and alopecia (1,2).

In addition to its cytotoxic effects, taxol has also been shown to be a potent inhibitor of angiogenesis (3). Cancer may be considered to be an angiogenesis-dependent disease because cancerous tumors, through the release of angiogenic factors, induce new blood vessel growth, or neovascularization, in order to sustain their continued growth (4). Taxol has been shown to inhibit many of the steps involved in angiogenesis, such as cell proliferation, cell migration, and collagenase secretion (5). The combination of both cytotoxic and anti-angiogenic properties may result in a more effective agent to treat cancer.

About 64% of all cancer patients present with localized disease. Following initial treatment of patients with localized disease, it has been estimated that 32% will have a recurrence of the disease and of these, 66% will relapse due to local recurrence of the disease compared to 34% who will relapse due to distant metastases of the disease (6). Local recurrence of tumors generally occurs near the previous surgical excision site of the primary tumor. In studies of recurrence patterns of glioblastoma multiforme and anaplastic astrocytoma, it was found that 90% of all recurrences are located within a 2 cm margin of the primary tumor (7,8).

We are developing drug loaded polymer formulations which we term "surgical pastes". The application for these surgical pastes would be at a tumor resection site where drug would be released locally over a period of weeks to months to prevent local recurrence of the disease. The polymer base of this surgical paste is poly(ϵ-caprolactone) (PCL).

PCL is a biocompatible, biodegradable polymer which either alone, or with other biocompatible polymers, has been investigated for various uses including drug delivery devices (9,10), nerve guides (11), and artificial skin (12). PCL has a long biodegradation lifetime in the order of 6 to 9 months (13, 14) and is therefore potentially suitable for the surgical paste formulation in terms of providing a slow release of drugs from the matrix.

Taxol has been incorporated into blends of PCL and methoxypolyethylene glycol (MePEG) which have a low melting point (onset of melting is 45°–50°C). Following gentle warming, the molten taxol-polymer material may be delivered from a syringe directly to the tumor resection site where it solidifies at 37°C to form a waxy solid.

The objective of this work was to characterize PCL and blends of PCL with MePEG to be used as the basis of a surgical paste formulation. The *in vitro* release rates of taxol from these pastes were investigated and the antiangiogenic activity of taxol released from surgical paste was assessed using a chick chorioallantoic membrane (CAM) bioassay.

METHODS

Materials

Taxol was supplied by Hauser Chemicals, Boulder, CO. Two sources of poly(ϵ-caprolactone) were used. Polysciences (Warrington, PA) supplied PCL labelled to be of molecular weight between 10 and 20,000 g/mol (PCL-PS), and Birmingham Polymers Inc. (Birmingham, AL) supplied PCL with a labelled density of 0.27 g/dl (PCL-BPI). Methoxypolyethylene glycol (MePEG), MW 350, was from Union Carbide (Danbury, CT).

Acetonitrile (ACN), methanol, dichloromethane (DCM), and chloroform were all HPLC grade from Fisher Scientific (Fairlawn, NJ). All buffers were made using deionized distilled water.

[1] Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, B.C., Canada.

[2] Angiogenesis Technologies Inc., Vancouver, B.C., Canada.

[3] To whom correspondence should be addressed at Faculty of Pharmaceutical Sciences, U.B.C., 2146 East Mall, Vancouver, B.C., Canada, V6T 1Z3.

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Experimental

Gel Permeation Chromatography

The molecular weights of the PCL polymers were determined at ambient temperature by gel permeation chromatography with a Shimadzu LC-10AD HPLC (Kyoto, Japan) through a 10^4 Å Hewlett Packard PLgel column and equipped with a Shimadzu RID-6A refractive index detector (Kyoto, Japan). The mobile phase was chloroform flowing at a rate of 1 mL/min. The sample was 20 μL of a 0.2% solution of polymer. In this method, the molecular weight was calculated using universal calibration with polystyrene standards in the range of 17.5k and 300k g/mol from Pressure Chemical Company, Pittsburgh, PA.

A Canon-Fenske viscometer was used to measure viscosity of 0.2% solutions of PCL in chloroform at a temperature of 25° ± 0.01°C. Intrinsic viscosities of polymer samples were then calculated using the approximation of Solomon and (15) Ciuta. All measurements were done in triplicate.

Preparation of Surgical Paste

Taxol was blended with molten PCL samples at 60°C and loading levels of 1 to 30% taxol. When MePEG was used in the formulation the taxol was first blended with the MePEG before this mix was incorporated into molten PCL such that final taxol loadings were between 1 to 30% and the PCL:MePEG ratio was 4:1. The molten pastes were allowed to cool to a solid at room temperture.

Taxol was also incorporated into polymer blends by solvent casting. Between 1 and 30% taxol was combined with either PCL-PS or PCL-BPI so that the total quantity was about 100 mg. This mix was dissolved in 1 mL DCM. The solution was heated to 70°C in a water bath and the DCM was evaporated with mixing until a solid paste was formed. The paste was stored uncovered overnight at room temperature to allow evaporation of DCM. To investigate the effectiveness of the drying process in removing DCM from the paste, the residual DCM was determined by thermogravimetric analysis (TGA) using a TA Instruments TGA 51 Thermogravimetric Analyzer on the 1% and 30% samples of solvent cast surgical paste from both sources. Samples were heated from 30° to 200° C at 10°C per minute and weight loss was found to be less than 0.1%.

A sample of 20% taxol in PCL:MePEG (4:1) was sterilized using 30 KGy gamma irradiation from a Co-60 source (Nordion International, Kanata, Ontario).

Thermal Analysis

Differential scanning calorimetry (DSC) was done with a TA Instruments 2000 controller and Dupont 910S DSC (Newcastle, Delaware) using crimped aluminum pans holding between 1.7 and 3.3 mg of sample and a heating rate of 10°C/min. DSC scans were used to analyze the thermal characteristics of taxol, PCL (both sources), 30% taxol loaded PCL-PS, PCL-PS blended with MePEG (4:1), and taxol loaded into PCL-PS:MePEG (80:20) polymer blends at taxol concentrations between 1 and 30%. The effect of sterilization with 30 KGy of gamma irradiation on the thermal properties of 20% taxol in PCL-PS:MePEG (4:1) was determined using 5.0 mg samples of irradiated and non-irradiated materials. The effect of MePEG on the melting point of PCL-PS was determined by DSC using a DuPont Series 99 thermal analyzer and 910 DSC in a N_2 atmosphere at 20 psi and a scanning rate of 2.5°C per minute. A Mettler FP2 hot stage microscope was used to confirm thermal events of PCL-PS and taxol, as a 30% taxol in PCL-PS formulation was heated at a rate of 10°C/min.

Strength Testing

A CT-40 mechanical strength tester (Nottingham, England) interfaced with an Apple II Plus computer was used to measure the effect of MePEG on the strength of PCL-PS. PCL-PS containing 0, 5, 10, or 20% MePEG were melted and cast as tablets with a diameter of 0.88 cm and a thickness of 0.560 cm. The ability of these tablets to withstand a diametrically applied force was measured by the CT-40 apparatus.

Swelling Studies

Samples of PCL-PS containing between 0 and 20% MePEG were heated to 60°C and cast in moulds to produce paste cylinders with of diameters 7.8 mm and height 3.9 ± 0.4 mm. The cylinders were stored at 37°C in 10 mL of 10 mM phosphate buffered saline, pH = 7.4 (PBS) containing albumin (0.4 g/mL). At various times the cylinders were removed from buffer, blotted dry, weighed, and returned to buffer. Percent weight change over time was recorded as was the percent volume change in each cylinder at the completion of the experiment. When the samples reached constant weight they were weighed and placed in a silica gel desiccator under vacuum to dry to constant weight. The difference between sample weights before and after desiccation was taken as water loss from the polymer.

Scanning electron micrographs (SEM) were taken of the interior of PCL-PS and PCL-PS:MePEG (4:1) pellets which had been swelled in PBS then dried in a silica gel vacuum desiccator to constant weight. Samples were mounted on graphite studs using double sided tape and colloidal graphite in alcohol (DAG 154, Acheson Colloids Ltd., Brantford, Ontario) and coated with a 100 Å film of gold-paladium (60:40) using a Hummer Sputter Coater. Images were taken using a Hitachi SEM (S-2300, Tokyo, Japan) with 5 kV beam at a magnification of 5000 × and downloaded to a computer using Quartz PCI-image management system software.

In Vitro Release Studies

Accurately weighed circular-shaped, taxol loaded surgical paste pellets of about 2.5 mg were tumbled end-over-end at 20 rpm at 37°C in PBS containing albumin (0.4 mg/mL), within glass Kimax tubes with PTFE lined screw caps. At various time intervals supernatants were removed for taxol analysis and replaced with fresh buffer at 37°C.

Taxol was extracted by adding 1 mL of DCM to the buffer and shaking the tube for 5 seconds to allow the taxol to partition into the organic phase. The aqueous supernatant was discarded and the organic phase was dried at 60°C under a stream of N_2 gas. The residue was dissolved in 1 mL of a 60:40 mix of ACN and water. This solution was centrifuged at 1000 g to pellet the albumin in solution and the supernatant was transferred to HPLC sample vials. HPLC analysis was performed using a 110A pump and C-8 ultrasphere column (Beckman) with

dimensions of 5 μm × 4.6 mm × 25 cm, and a SPD-6A uv detector set at 232 nm, a SIL-9A autoinjector and C-R3A integrator (Shimadzu). The injection volume was 20 μl and the flow rate was 1.0 mL/min. Under these conditions the taxol peak eluted at 6.2 minutes and the amount of taxol present in the sample was calculated from the standard curve. The standard curve was linear over the range of taxol concentrations studied (2–20 μg/mL) and had an r^2 value of 0.999.

Release rate profiles for taxol were obtained from PCL, PCL-PS:MePEG blends (4:1), and irradiated PCL-PS:MePEG blend at different taxol loadings between 1–30%. The amounts of taxol released (μg) were normalized for 2.5 mg surgical paste samples and the results are expressed as mean ± SD for 4 separate tubes.

CAM Bioassay

This assay was performed as described by Dugan *et al.* (16) with some modifications. Fertilized, domestic chicken eggs from Fitzsimmons Consulting and Research Services (Surrey, BC, Canada) were incubated (Humidaire model 21 incubator, New Madison, OH) for four days prior to shell-less culturing. The egg contents were incubated at 37°C, 90% relative humidity and 3% CO_2 for 2 days (Forma Scientific model 3158 incubator, Marietta, OH). On day 6 of incubation 3 mg aliquots of taxol loaded or control (taxol free) surgical paste were placed directly on the CAM surface. After a 2 day exposure the vasculature was examined using a stereomicroscope fitted with a Contax 35 mm camera. Antiangiogenic activity was defined as the presence of an avascular zone measuring at least 4 mm in diameter surrounding the paste pellet.

Paste samples of PCL loaded with between 0.05–20% taxol were tested to compare their ability to inhibit angiogenesis on the CAM.

RESULTS

Two sources of PCL (PCL-PS and PCL-BPI) were obtained, labelled to be of similar molecular weights. Both sources were characterized to determine whether there were any differences in either physicochemical properties or taxol release from the PCL samples.

Prior to selection of MePEG (molecular weight, 350) for blending with PCL, test blends of PCL with MePEG (M.W. 350, 550, and 750), PEG (M.W. 200, 1500, and 8000) and propylene glycol were prepared. We found that MePEG 350 blended homogeneously with PCL and reduced the PCL melting point.

Gel permeation chromatography was used to determine the molecular weights of PCL samples (both sources). The intrinsic viscosities of the PCL samples were measured to be 0.34 ± 0.02 and 0.27 ± 0.01 poise for PCL-PS and PCL-BPI respectively. Figure 1 gives the GPC chromatograms of the PCL samples with their molecular weights. The chromatogram of PCL-PS showed a double peak indicating a bimodal molecular weight distribution since polymers of different molecular weight show different retention times. Therefore the double peak indicated the presence of two molecular weight groups within the PCL-PS sample. Gamma irradiation at 30 kGy did not affect the molecular weight of the PCL-PS:MePEG blend.

All DSC scans consisted of a single melting endotherm. Table I gives melting points (peaks of the melting endotherms) and ΔH of PCL melting in representative scans as well as a notation of the relative widths of the peak. Taxol had a melting point of 221°C with an enthalpy of melting of 59.34 J/g. Surgical paste samples containing 20% taxol in PCL-PS:MePEG (4:1) which had been irradiated did not show different thermal properties from a sample of surgical paste from the same batch which was stored at room temperature but was not irradiated. The control and irradiated samples of surgical paste showed peak melting endotherms at 56.2° and 55.9°C and enthalpies of melting of 83.41 and 82.88 J/g respectively. Both sources of PCL had melting points of 52°C, with the PCL-PS sample showing a lower enthalpy of melting, 58.53 J/g compared to 66.28 J/g for the PCL-BPI sample. The melting peak of PCL-PS contained a shoulder which can be explained by the bimodal molecular weight distribution of PCL-PS. A blend of PCL-PS:MePEG (4:1) melted at 50.4°C and had an enthalpy of melting of 71.00

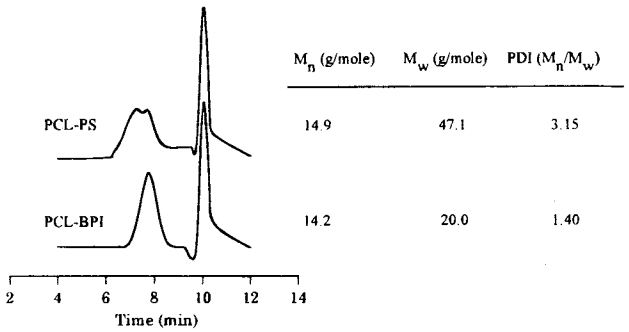

Fig. 1. Chromatograms of gel permeation chromatography scans of PCL-PS and PCL-BPI. Scans were performed using 20 μL injections of 0.2% polymer solutions in chloroform using a chloroform mobile phase at a rate of 1 mL/minute. The molecular weights reported were calculated from polystyrene standards.

Table I. Thermal Data Generated from Differential Scanning Calorimetry Scans of Taxol and Selected Thermopaste Samples Using Crimped Open Aluminum Pans and a Heating Rate of 10°C/min

Sample	Melting point (°C)	ΔH of taxol or PCL melting endotherm (J/g)
Taxol	221.2	59.34[c]
20% taxol in PCL-PS:MePEG (4:1)	56.2	83.41[c]
20% taxol in PCL-PS:MePEG (4:1), irradiated sample[a]	55.9	82.88[c]
PCL-PS	52.0	58.53[d]
PCL-BPI	52.3	66.28[c]
PCL-PS:MePEG (4:1)	50.4	71.00[c]
30% taxol in PCL-PS[b]	48.5 ± 1.9	74.8 ± 6.9[f]
30% taxol in PCL-PS:MePEG (4:1)[b]	49.9 ± 2.1	94.1 ± 10.1[c]

[a] Sterilized using gamma radiation dose of 30 KGy.
[b] Scans were run to 250° C but no peak for taxol was observed. Mean ± 1 SD for n = 3.
[c] Symmetrical peak.
[d] Asymmetrical peak (shoulder).
[e] Asymmetrical peak (small shoulder).
[f] Asymmetrical peak (broad).

J/g. Although only shown to 65°C, scans of surgical paste containing taxol were run to 250°C but no peak was seen due to the melting of taxol. Analysis by hot stage microscopy revealed that taxol exists as needle shaped crystals in the paste at room temperature but dissolved slowly in the PCL-PS between 100 and 200°C as the sample was heated. The incorporation of taxol slightly decreased the melting point of the polymer paste. The amount of taxol in the PCL-PS:MePEG blends did not affect the location of the peak of the melting endotherm (~51°C) within a range of 1–30% taxol. It was noted that samples containing between 10–30% taxol in PCL-PS:MePEG blends had a step transition which occured at 31°C which has not been explained.

The effect of MePEG on the melting point of PCL was investigated using DSC at a heating rate of 2.5°/min. The incorporation of 0, 1, 2, 5, 10, 20, and 30% MePEG decreased the onsets of melting of PCL-PS from 48.5°C (0% MePEG) to 47.6°, 47.5°, 46.6°, 45.8°, 44.3° and 43.4°C respectively.

The tensile strength of PCL was decreased by the addition of MePEG to the polymer in a concentration dependent manner. With the addition of 5, 10, and 20% MePEG to PCL-PS the tensile strength was reduced from 179.4 ± 34.2 to 151.6 ± 29.5, 85.8 ± 20.6, and 26.7 ± 2.7 N/cm^2 respectively. As MePEG loadings were increased the blends became softer and the cylinders had an increased ability to deform before failure.

Cylinders of PCL stored in PBS increased in weight by 0.8 ± 0.4% indicating minimal swelling. The samples containing PCL with MePEG lost weight due to the diffusion of water soluble MePEG out of the polymer blend into the aqueous buffer. Weight loss was 1.8 ± 0.6%, 3.3 ± 0.5%, and 5.8 ± 1.1%, for the samples containing 5, 10 and 20% MePEG in PCL respectively. The PCL cylinders showed insignificant volume changes while the cylinders containing MePEG decreased in volume by 3.2%, 7.7%, and 6.2% for the 5, 10 and 20% MePEG samples respectively. These volume changes were all significantly different from zero at $p < 0.025$ using a paired two sample t-test. Following swelling the samples initially loaded with 0, 5, 10, and 20% MePEG were found to have contained 0.2 ± 0.4%, 3.7 ± 0.7%, 8.5 ± 1.2%, and 18.6 ± 0.6% water respectively. SEM images of the interior of "dried" samples of PCL-PS which had contained 0 and 20% MePEG are shown in Figure 2 at a magnification of 5000×. The water channels produced in the 20% MePEG sample are clearly visible.

The cumulative *in vitro* release profiles of taxol, 1–30% loading, from PCL-BPI and PCL-PS paste samples which had been solvent cast are shown in Figure 3 (A and B respectively). Taxol release was characterized by an initial burst phase lasting 1 or 2 days followed by a period of sustained slow drug release. In both cases the amount of taxol released was significantly lower for the samples containing 1% taxol than for the samples of higher taxol loading. The samples of PCL-PS loaded with between 5 and 30% taxol loading showed similar taxol release profiles (from 2.5 mg surgical paste samples) of between 30 and 40 μg taxol released after 20 days (Figure 3A). When PCL-BPI was used as the polymer vehicle (Figure 3B) the same pattern was seen for taxol release. The taxol release from the 30% taxol loaded sample was significantly higher (Student's t-test, $p < 0.05$) than for the samples containing 5, 10, or 20% taxol. With the exception of 30% taxol loading, surgical paste samples made from either PCL-PS or PCL-BPI did not show significant differences in the cumulative amount of taxol released when compared at equal taxol loading levels.

The taxol release profiles (1–30% loading) from PCL-PS and PCL-PS:MePEG (4:1) are shown in Figure 3 (C and D respectively). Taxol again shows biphasic release characterized by a burst phase followed by a period of slow sustained release. The 1% taxol sample in PCL-PS released significantly less taxol than did the samples containing higher loading levels of taxol. At higher loadings (20–30%) the release of taxol from the PCL-PS formulations was inhibited by the presence of MePEG. Taxol release from the PCL-PS surgical paste was an average of 1.5 times higher than from the PCL-PS:MePEG formulations. At both loading levels this difference was significant at $p < 0.05$ using the Student's t-test for two samples.

A comparison of the release of 20% loaded taxol from PCL-PS:MePEG (4:1) paste from irradiated and non irradiated samples is shown in Figure 4. After 20 days the cumulative taxol released from both samples were not different from each other and was similar to that observed in previous formulations of 20% loaded surgical paste (Figure 3D). The curves did not however, show the biphasic release seen with other surgical paste formulations but instead gave steady taxol release over the 20 day course of the study.

Figure 5A and B shows CAM images of control PCL-PS paste and 5% taxol in PCL paste respectively. The control PCL-PS paste did not affect the vasculature of the CAM (Figure 5A) while the taxol loaded sample induced an avascular zone in the treated region of the CAM measuring at least 4 mm in diameter (Figure 5B). The taxol induced avascular zone typically contained areas of altered blood flow continuity, disrupted vascular networks, and sparse blood vessel remnants. At the periphery of this zone, blood vessels are characterized by their altered "elbowing" appearance. The antiangiogenic activity of taxol released from paste samples loaded with between 0.05 and 20% taxol is given in Table II. Inhibition of angiogenesis was observed for all formulations containing 0.25% taxol and greater (7.5 μg of taxol and greater). Surgical paste loaded with 0.1% (3.0 μg) taxol showed only partial angiogenesis inhibition with only 1 of 8 samples giving a positive result for antiangiogenic activity. No antiangiogenic activity was seen for surgical paste samples loaded with 0.05% taxol.

DISCUSSION

Analysis of the two sources of PCL revealed that, although they had a similar M_n, the PCL-PS had a wider molecular weight distribution than did the PCL-BPI with the PCL-PS exhibiting a bimodal molecular weight distribution. The different molecular weight chains may have resulted in the formation of two different crystal forms which melted at slightly different temperatures. In these formulations the taxol is dispersed within the polymer as needleshaped crystals since it is only when heated to 200°C that the dispersed taxol phase had completely dissolved in PCL-PS. The heat of fusion of 100% crystalline PCL is reported to be 139.5 J/g and this value can be used to calculate the degree of crystallinity of a PCL sample using the area under the melting endotherm on a DSC scan (9). Using this value the PCL-PS sample had a degree of crystallinity of 42% while the PCL-BPI sample was 47.5% crystalline. When MePEG was blended with PCL-PS the melting endotherm of the PCL-PS had an area corresponding to 71.0 J/g (50.9%

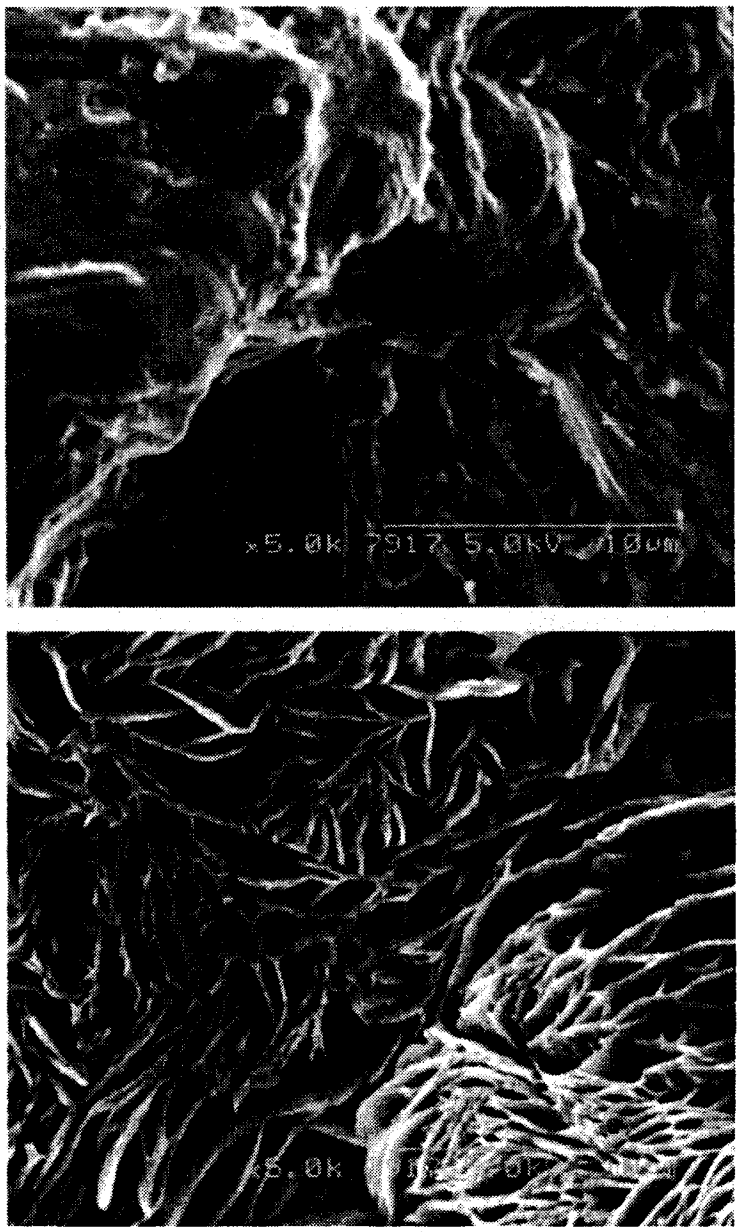

Fig. 2. Scanning electron microscope images of the interior of A: PCL-PS pellet and B: pellet which initially consisted of 20% MePEG in PCL-PS, which had been stored in PBS buffer for 15 days before being dried to constant weight (Mag = 5000×).

crystallinity). A higher degree of crystallinity of a polymer sample can be achieved by increasing the mobility of the polymer chains as well as the time available for crystallization (17,18). The increase in PCL-PS crystallinity may be the result of MePEG becoming incorporated between the PCL-PS chains and acting as a plasticizer, giving the PCL-PS chains greater mobility and allowing them to achieve a greater degree of crystallinity. Furthermore the reduction of the melting point of the MePEG:PCL-PS blend relative to PCL-PS alone may give the blend more time to solidify when cooled from a melt resulting in an increased time for crystallization. The lower melting point of PCL-PS seen when the effect of MePEG on melting point was measured, compared to the data in Table I, is due to the values being measured as the onset of melting rather than the peak of the melting endotherm, and the use of a lower heating rate of 2.5° per minute.

Taxol release from PCL-PS and PCL-BPI were not different after 20 days as revealed in Figure 3 showing that small differences in the molecular weight of PCL samples do not have an appreciable effect on the release characteristics of taxol *in vitro*. Visual evidence using an optical microscope suggests that at taxol loadings of 1%, the taxol is completely dissolved in the surgical paste and forms a monolithic solution but at higher loadings, 5% and up, taxol is present in the polymer as monolithic dispersions. As a result the mechanism of taxol release from samples loaded with 1% taxol is different from that of 5 to 30% loaded samples of surgical paste. Studies by Pitt *et al.* (10) showed that release of steroids from PCL, which

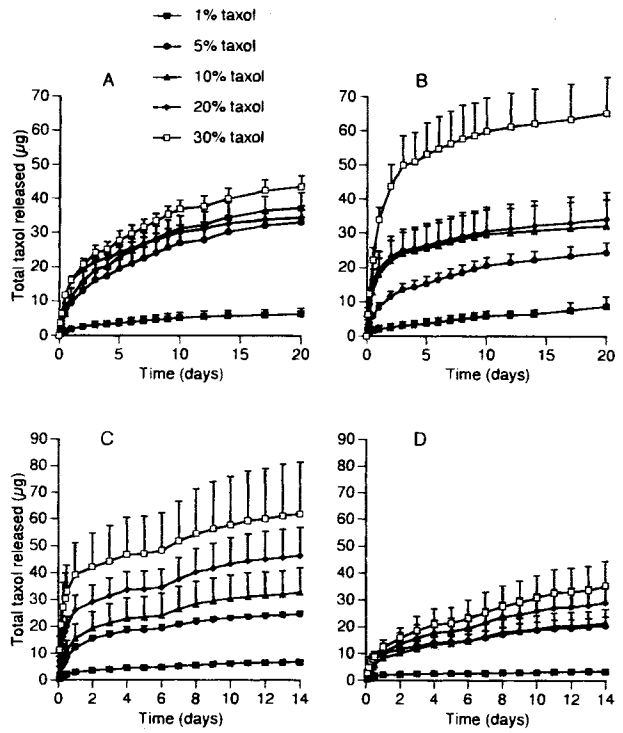

Fig. 3. Taxol release profiles from A: PCL-PS (Polysciences, Warrington, PA); B: PCL-BPI (Birmingham polymers, Birmingham, AL.); C: PCL-PS; and D: PCL-PS:MePEG blend (4:1) at various taxol loadings. The polymer samples in A and B were solvent cast while the polymer samples in C and D were made without solvent. All release curves were normalized to a 2.5 mg weight. Error bars are shown in the positive direction only and indicate 1 S.D. of four samples.

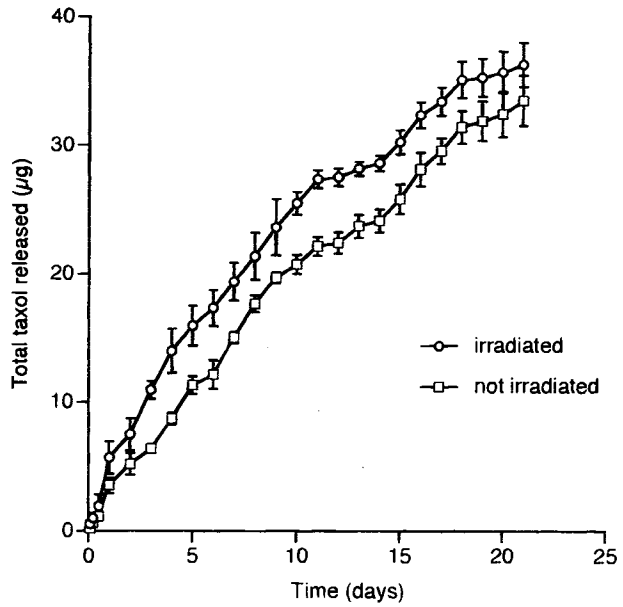

Fig. 4. Effect of sterilization by gamma irradiation (30 KGy) on the release profile of 20% taxol loaded PCL-PS:MePEG (4:1). All release curves were normalized to a 2.5 mg weight. Error bars indicate 1 S.D. of four samples.

is only partially crystalline, was diffusion controlled. Taxol has a very low water solubility however (19) and resists diffusion from the hydrophobic polymer to the aqueous medium. Since PCL does not begin to erode in twenty days we speculate that taxol release is achieved through the formation of water channels similar to the mechanism for release of macromolecules from ethylene vinyl acetate polymers as described by Langer *et al.* (20, 21). Uptake of water by PCL is minimal, as described in this study. This is particularly true in the crystalline regions of the polymer where diffusion of drug is also restricted (9). The result is that taxol release from PCL is characterized by an initial burst phase lasting 1 or 2 days due to release of taxol associated with the surface of the polymer pellets followed by a period of very slow release, when taxol release rate is limited by the penetration of water into the PCL.

Figure 3 shows the effects of blending MePEG with PCL on taxol release. It was thought that the relatively hydrophilic MePEG would dissolve into the aqueous medium and open channels within the PCL matrix through which water could penetrate and taxol could diffuse out. Although we have demonstrated that MePEG diffused out of the polymer matrix and enhanced water uptake by PCL-PS this did not lead to an increase in the *in vitro* taxol release from the polymer. A similar observation was made by Sturesson *et al.* (22) who were studying the release of timolol maleate from poly(lactic-co-glycolic) acid. In this study the addition of polyethylene glycol (PEG) inhibited the secondary burst phase of release (erosion of polymer matrix) and caused a constant release rate. The PCL-PS heat of melting from triplicate samples of 30% taxol in PCL-PS or PCL-PS:MePEG showed a statistically significant increase in PCL-PS crystallinity in the samples containing MePEG (one-tailed t-test, $p < 0.03$). Increasing the crystallinity of PCL results in a polymer which will degrade more slowly and have a decreased diffusion coefficient due to an increased tortuosity of the diffusional pathway (9). If the rate limiting step in the release of taxol from PCL is the formation of water channels in the polymer matrix it is interesting to speculate that an increase in PCL-PS crystallinity brought about by the incorporation of MePEG may also retard water uptake by the PCL-PS regions containing taxol and result in a corresponding decrease in the rate of taxol release.

The CAM assay has been shown by Folkman (23) to be a rapid method of testing agents for antiangiogenesis activity and has been used to assess antiangiogenic activity of other anticancer agents (24). The results from this assay show that taxol is released from the paste formulation in an active form and demonstrates antiangiogenic activity at almost all doses tested. No effect of loading levels can be seen since the CAM assay gives only a positive or negative response based on the definition of avascular zone size.

Sustained release of anticancer drugs from polymers (25) and taxol from biodegradable polymers (26) have been investigated recently. Intracranially administered, 10 mg biodegradable polyanhydride discs containing taxol (20–40% loading) were found to increase the survival time of rats with intracranial malignant glioma by 2 or 3 times over control rats. In order to develop a useful polymeric delivery system for taxol we need to be able to control or manipulate the properties of the system both in terms of its physical and release characteristics.

With the addition of MePEG, both the thermal and physical properties of PCL are altered. The decreased melting point

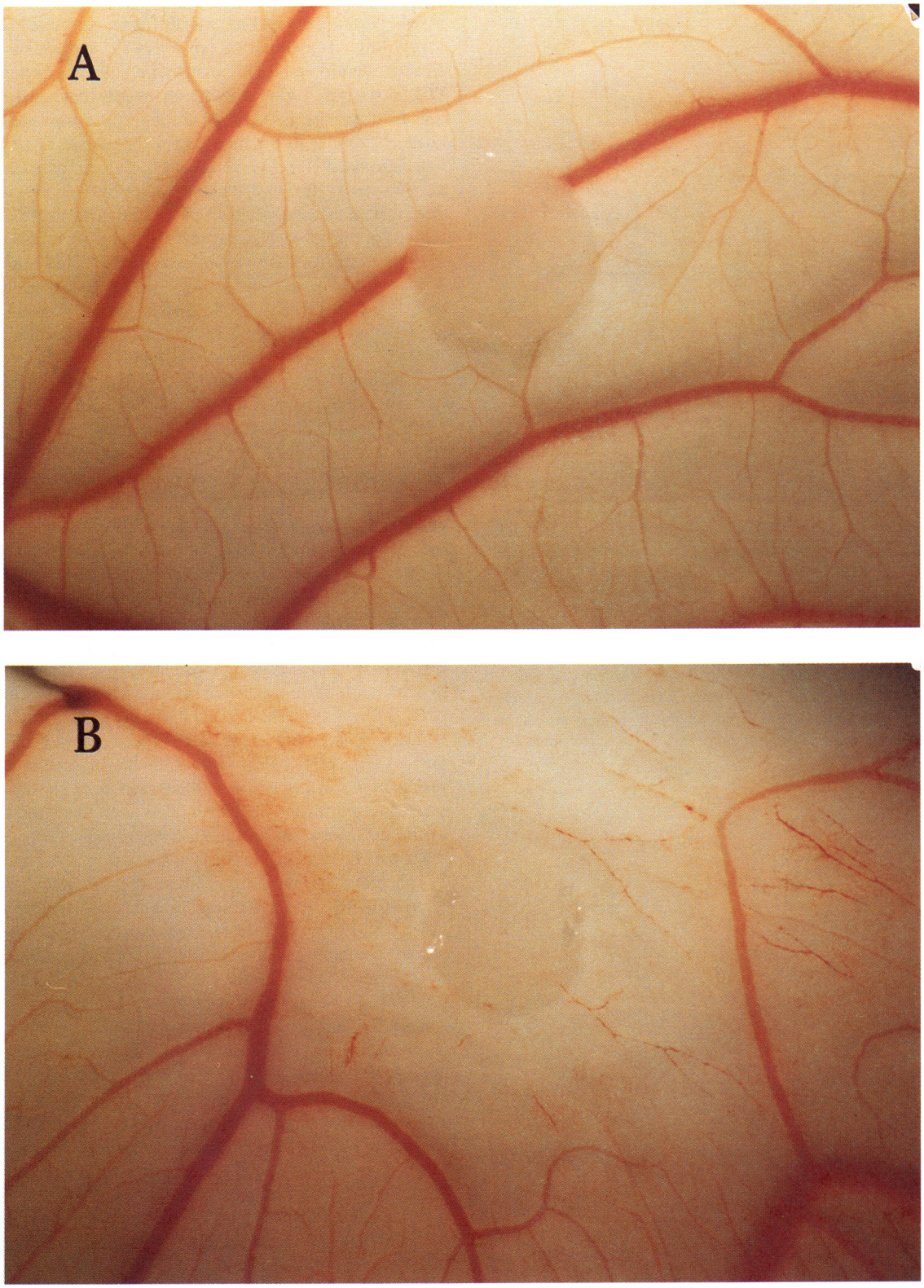

Fig. 5. Photographs showing effect of 3 mg surgical paste disks on chick chorioallantoic membrane (CAM). A: Control surgical paste (taxol-free) on living, unstained CAM showing normal blood vessel architecture of CAM located about PCL surgical paste disk. Mag = 10×. B: 5% taxol loaded surgical paste on living unstained CAM. After 48 hour period, this treatment induced an avascular zone measuring approximately 6 mm in diameter characterized by blood stasis and vessel disruption. Mag = 8×.

Table II. Inhibition of Angiogenesis by Taxol Loaded Thermopaste Using the Chick Chorioallantoic Membrane Assay

% Taxol	Dose of taxol (μg)	Embryos evaluated (+/n)[a]
0.05	1.5	0/9
0.1	3.0	1/8
0.25	7.5	4/4
0.5	15	4/4
1	30	8/8
5	150	4/4
10	300	5/5
20	600	6/6
Control	0	0/30

[a] Positive angiogenesis inhibition defined as avascular zone surrounding thermopaste pellet of at least 4 mm in diameter.

results in a formulation which can be applied at lower temperatures and remains liquid longer than would PCL alone. The reduction in tensile strength of PCL brought about by the plasticizing effect of MePEG gives a polymer which can be more easily manipulated than PCL which cools to a brittle solid.

We have developed a sustained release delivery system for taxol from PCL. Using additives we have been able to adjust the physical characteristics of the surgical paste while maintaining a system for sustained taxol release. Although based on only a single formulation study, we have preliminary evidence that the surgical paste formulation can undergo sterilization by gamma irradiation without affecting its thermal and release characteristics. Studies to assess the *in vivo* activity of taxol loaded surgical paste are underway.

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