The AAPS Journal 2004; 6 (3) Article 27 (http://www.aapsj.org).

Development and Characterization of Biodegradable Chitosan Films for Local Delivery of Paclitaxel

Submitted: January 23, 2004; Accepted: June 22, 2004; Published: October 11, 2004.

Anand Babu Dhanikula,[1] and Ramesh Panchagnula[1]

[1]Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), SAS Nagar 160062, India

ABSTRACT

Intratumoral and local drug delivery strategies have gained momentum recently as a promising modality in cancer therapy. In order to deliver paclitaxel at the tumor site in therapeutically relevant concentrations, chitosan films were fabricated. Paclitaxel could be loaded at 31% wt/wt in films, which were translucent and flexible. Physicochemical characterization of paclitaxel via thermal, spectroscopic, x-ray diffraction, and electron microscopy techniques revealed information on solid-state properties of paclitaxel as well as chitosan in films. While chitosan was in amorphous form, paclitaxel seemed to be present in both amorphous and crystalline forms in film. The polymeric dispersion of paclitaxel in poloxamer formed fibrous structures generating discontinuities in the film matrix, thereby leading to the introduction of perturbations in the packing arrangement of polymer chains. These films released only 10% to 15% of loaded paclitaxel by a burst effect under in vitro testing conditions, with lysozyme having no effect on the release. However, films softened after implantation in mice and lost integrity over time. The implantable delivery system is not only biodegradable but also well tolerated in vivo and hence, biocompatible as revealed by histological studies. The lack of formulation-induced local inflammatory responses of paclitaxel chitosan films suggests a new paradigm for localized chemotherapy based on implantable systems.

KEYWORDS: paclitaxel, local delivery, film, solid-state, histology, mice.

INTRODUCTION

In recent years, biodegradable polymeric systems have gained importance for design of surgical devices, artificial organs, drug delivery systems with different routes of administration, carriers of immobilized enzymes and cells, biosensors, ocular inserts, and materials for orthopedic applications.[1] These polymers are classified as either synthetic (polyesters, polyamides, polyanhydrides) and natural (polyamino acids, polysaccharides).[2] Polysaccharide-based polymers represent a major class of biomaterials, which includes agarose, alginate, carageenan, dextran, and chitosan.

Chitosan, β(1,4)2-amino-2-D-glucose, is a cationic biopolymer produced by alkaline N-deacetylation of chitin, which is the main component of the shells of crab, shrimp, and krill. Chitosan has found many biomedical applications, including tissue engineering, owing to its biocompatibility, low toxicity, and degradation in the body by enzymes such as chitosanase and lysozyme,[3] which has opened up avenues for modulating drug release in vivo in the treatment of various diseases. These chitosan-based delivery systems range from microparticles to nanoparticles[4] to gels[5] and films.[6] Further, gels and films of chitosan have been used for oral delivery of chlorhexidine digluconate in the treatment of fungal infections.[7] In addition, chitosan has been extensively evaluated as a carrier of various antineoplastic agents such as 5-fluorouracil,[8] mitoxantrone,[9] cytarabine,[10] and paclitaxel.[11]

The film-forming property of chitosan has found many applications in tissue engineering and drug delivery by virtue of its mechanical strength and rather slow biodegradation.[12] Some drug-loaded chitosan films are emerging as novel drug delivery systems,[13-14] and films appear to have potential for local sustained delivery of cancer chemotherapeutic agents. Following surgical removal of tumor, these implantable systems may be placed in the resection cavity to elicit a local response at the biophase; further, they may be secured by suturing at the site to prevent any displacement problems.

Though paclitaxel is the most extensively investigated anticancer drug in the last 3 decades, it is not a good option for the treatment of brain tumors after systemic administration.[15] Successful treatment of malignant brain tumors is alarmingly negligible because antineoplastic agents, including paclitaxel, have limited access to the tumor site across the blood brain barrier when administered systemically. An alternative approach to systemic delivery of antineoplastic drugs is localized delivery from a polymer matrix. In the field of local delivery, carmustine-loaded Gliadel wafer (Guilford Pharmaceuticals, Baltimore, MD) fabricated from poly(carboxyphenoxypropane:sebacic acid) proved very promising in

Corresponding Author: Ramesh Panchagnula, Department of Pharmaceutics, National Institute of Pharmaceutical, Education and Research (NIPER), Sector 67, Phase X, SAS Nagar, 160062 (Punjab) India. Tel: 91-172-2214682, 2214687. Fax: 91-172-2214692. Email: panchagnula@

clinical trials for the treatment of malignant glioma, increasing both survival and safety.[16] The objective of this study was to develop a chitosan film-based local delivery system for sustained release of paclitaxel to tumor site after implantation. These films have been evaluated for the release of impregnated paclitaxel, characterized by physical techniques and microscopy, and examined for inflammatory reactions by histological examination after implantation in mice.

Materials and Methods

Materials

Paclitaxel was a gift sample from Dabur India Ltd (Uttar Pradesh, India). Radioactive paclitaxel (^{14}C) of specific activity 42.5 mCi/mmol, chitosan (≥85% deacetylated), lysozyme, and Tween 80 were purchased from Sigma (St Louis, MO). Poloxamer 407 was obtained as gratis sample from BASF (Ludwigshafen, Germany). Soya phosphatidylcholine and phosphatidylglycerol were kindly provided by Nattermann & Cie Gmbh (Cologne, Germany). Absolute ethanol (ETOH) was procured from Merck KgaA (Darmstadt, Germany). Glycerol and glacial acetic acid were obtained from LOBA Chemie (Mumbai, India). High pressure liquid chromatography (HPLC)-grade methanol was obtained from (J.T. Baker, Madero, Mexico). Thiopentone sodium and gentamicin were of parenteral grade. All other reagents were analytical or reagent grade. Water obtained from ELGA purification unit (Marlow, UK) was used throughout the study. All animal experimentation was performed in accordance with protocols approved by the institutional animal ethical committee.

Preparation of Paclitaxel Chitosan Films

Because paclitaxel is a hydrophobic drug, 2 different approaches were used for its incorporation into chitosan films: one involved only phospholipids and the other used poloxamer 407 in presence of ETOH. Initially, a 10 mg/mL chitosan solution was prepared in 1% (vol/vol) acetic acid, and glycerol was included as a plasticizer at a chitosan:glycerol weight ratio of 2:1. In the first method, liposomes containing paclitaxel (spiked with radioactive component) were prepared by film hydration method using phosphatidyl choline and phosphatidyl glycerol (9:1, soya origin) at 6 to 12 mol% drug loading and were subsequently dispersed in chitosan solution. Then, film was cast by pouring the mixture on a glass plate (area 45.5 cm^2) followed by drying under vacuum for 48 hours at 37°C. In the second method, required quantities of paclitaxel and poloxamer 407 were dissolved separately in 1 mL of ETOH and mixed together, and the ethanolic solution was added to chitosan solution and agitated to disperse paclitaxel. Subsequently, the homogeneous suspension was cast into film by the method described above and dried at 60°C for 12 hours. After preparation, all films were stored in airtight containers for further studies.

Stability of Paclitaxel During Film Preparation

The following procedure was used to assess the stability of paclitaxel during the film preparation process. The prepared films were extracted twice with a solvent mixture of 1:1 acetonitrile and ETOH (vol/vol); the extract was evaporated; the residue obtained was reconstituted in mobile phase; and an aliquot was injected onto HPLC column. Stability-indicating chromatographic method was adopted for this purpose (Waters Corp, Milford, MA).[17] The method consisted of a Symmetry C18 column (250 × 4.6 mm; 5 µm) run using a mobile phase of composition methanol:water (70:30 vol/vol) at a flow rate of 0.5 mL/min, a Waters pump (600 E), and eluants monitored with Waters photodiode array detector (996 PDA) at 227 nm.

Content Uniformity of Films

To ensure uniform distribution of paclitaxel in film, a content uniformity test was performed. Samples representing different regions within film were cut and weighed, and paclitaxel was extracted with a 1:1 solvent mixture of acetonitrile and ETOH (vol/vol) twice for 12 hours each time at room temperature. These extracts were pooled for liquid scintillation counting (EG&G Wallac, Turku, Finland).

Release Studies

A definite weight range of 10-15mg of film was cut and placed in a 1.5-mL capacity microcentrifuge tube containing 1 mL of release medium of the following composition at 37°C: phosphate buffered saline (140 mM, pH 7.4) with 0.1% sodium azide and 0.1% Tween 80. At predetermined time points, 100 µL of release medium was sampled with replacement to which 3 mL of scintillation cocktail was added and vortexed before liquid scintillation counting. The cumulative amount of paclitaxel released as a function of time was calculated. In addition, to simulate the in vivo conditions, release of paclitaxel in presence of lysozyme (2 mg/100 mL) was also studied.

Film Thickness

Film thickness was measured using a micrometer (Mitutoyo, Kanagawa, Japan) with the smallest possible unit measurement count of 0.01 mm.

Tensile Strength

The effect of paclitaxel on mechanical properties of chitosan films was assessed through a tensile strength test. Tensile

strength of film was measured using texture analyzer TA-XT2i (Stable Micro Systems, Surrey, UK) with the following acquisition parameters:

1. 2 mm/s prespeed
2. 1 mm/s test-speed
3. 10 mm/s postspeed with an acquisition rate of 50 points/s
4. 5 kg load cell

Film was secured with tensile grips, and a trigger force of 5 g was applied. The resulting profiles were analyzed using Texture Expert, Version 1.22 (Stable Micro Systems, Surrey, UK).

Solid-State Characterization

To study the molecular properties of paclitaxel and chitosan, the solid-state characterization was done by the application of thermal, infrared, x-ray diffraction, and microscopy techniques. During these studies, solid-state characteristics of paclitaxel and chitosan were compared with those of film to reveal any changes occurring as a result of film preparation.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) studies were performed with a Mettler Toledo 821^e thermal analyzer (Greifensee, Switzerland) calibrated with indium as standard. For thermogram acquisition, sample sizes of 1 to 5 mg were scanned with a heating rate of 5°C/min over a temperature range of 25°C to 300°C. In order to check the reversibility of transition, samples were heated to a point just above the corresponding transition temperature, cooled to room temperature, and reheated up to 300°C.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectra were obtained for paclitaxel, chitosan, blank films, and paclitaxel films on Nicolet Impact 410 (Nicolet Analytical Instruments, Madison, WI). Spectra of paclitaxel and chitosan were obtained using the potassium bromide disc method, while those of films were acquired directly. In each case, 100 spectra in the region of 400 to 4000 cm^{-1} were co-added with a resolution of 2 cm^{-1}.

Scanning Electron Microscopy

Paclitaxel samples and chitosan films were viewed using a Jeol scanning electron microscope (SEM), JSM 1600 (Tokyo, Japan) for morphological examination. Powder samples of paclitaxel and films were mounted onto aluminum stubs using double-sided adhesive tape and then sputter coated with a thin layer of gold at 10 Torr vacuum before examination (Jeol Fine Coat, ion sputter, JFC-1100). The specimens were scanned with an electron beam of 1.2 kV acceleration potential, and images were collected in secondary electron mode.

X-ray Diffraction Studies

Molecular arrangement of paclitaxel and chitosan in powder as well as in films was compared by powder x-ray diffraction patterns acquired at room temperature on a Philips PW 1729 diffractometer (Eindhoven, Netherlands) using Cu Kα radiation. The data were collected over an angular range from 3° to 50° 2θ in continuous mode using a step size of 0.02° 2θ and step time of 5 seconds.

In Vivo Implantation Studies

Biodegradation of films was studied in Swiss mice. Initially, mice were anesthetized with thiopentone sodium (40 mg/kg) and occasional light ether inhalation, and an incision was made in the back of the neck region with a scalpel. After incision, the implantation site was created by tunneling immediately beneath the skin, then films were inserted and the skin was sutured. To prevent infection, mice were given gentamicin (2 mg/kg, intraperitoneal route) every 4 days. For in vivo implantation purposes, film was prepared in a plastic mold of radius 1.15 cm (instead of glass plate) with each mouse receiving one such film.

Histology Studies

Histology studies were performed to examine the acute toxicity of film at the implantation site. After a 2-month implantation period, mice were humanely killed by cervical dislocation and an incision was made in the implantation area. Then, the tissue in which the film was imbibed was removed and stored in 50% formalin until processing. Subsequently, tissue processing involved dehydration through a graded series of alcohols (70%, 80%, 95%, and 100%), followed by xylene and then infiltration with paraffin. For obtaining thin sections (3-5 μm), tissues were embedded on the edge of paraffin blocks and were cut on a rotary microtome. These sections were deparafinized, rehydrated with graded alcohols (100%, 95%, 80%, and 75%), and stained with hemotoxylin/eosin for microscopic examination.[18] Similarly, sections of paclitaxel chitosan film and tissue of healthy mouse were obtained to serve as control.

RESULTS

Preparation of Films

The difficulty of incorporating water-insoluble paclitaxel molecules was circumvented by concomitant inclusion of unsatu-

Table 1. Composition and Thickness of Paclitaxel Chitosan Films Obtained by Casting Method*

Film Code	Chitosan (mg)†	Glycerol (mg)	Phosphatidyl Choline (mg)‡	Phosphatidyl Glycerol (mg)‡	Poloxamer 407 (mg)§	Paclitaxel (mg)	Film Thickness (μm)
FLM-1-PCL	200	100	130	15	-	10	50-60
FLM-2-PCL	200	100	130	15	-	15	50-60
FLM-3-PCL	200	100	130	15	-	20	50-60
FLM-4-PCL	150	100	-	-	50	10	40-45
FLM-5-PCL‖	25	10	-	-	10	20	100-120

*FLM indicates film. Films 1, 2, 3, and 4 were cast on glass plate of surface area 45.5 cm^2, while film 5 was cast in plastic mold of surface area 4.2 cm^2. Films 1, 2, and 3 were dried at 37°C; films 4 and 5 were dried at 60°C.

†Chitosan is ≥85% deacetylated.

‡Liposomes containing paclitaxel were prepared from phospholipids of soya origin by film hydration method and used without extrusion; encapsulation efficiency ~95%.

§The type of poloxamer used here could be replaced with other grades or other polymers which might lead to better formulation.

‖Formulation was selected for physicochemical characterization and histology studies (see text).

rated phospholipids (in the form of liposomes) or poloxamer along with paclitaxel in film (Table 1). Unsaturated lipids were used to prepare liposomes (as a means of incorporation of paclitaxel in film) since earlier studies have shown that these lipids yield best encapsulation efficiency of paclitaxel (D.A. and R.P., unpublished data, 2000). These films were obtained by casting method and appeared either transparent or translucent and were pale yellowish in color. Further, poloxamer-containing films showed good loading capacities of 20 mg of paclitaxel per 25 mg of chitosan, and these films had a mean weight of 63.5 mg. At this loading percentage (30%-31%), essentially all drug could be incorporated into film without any precipitation. A lower fabrication temperature of 37°C was chosen for lipid films in contrast to poloxamer films (60°C) in order to minimize hydrolysis and oxidation of unsaturated phospholipids. When films were examined for thickness, the lipid films (FLM-1,2,3-PCL) ranged from 50 to 60 μm, while poloxamer films FLM-4-PCL (cast on glass plate) ranged from 40 to 45 μm, and FLM-5-PCL (cast in plastic mold) ranged from 100 to 120 μm. At constant casting surface area, the higher thickness of lipid films (FLM-1,2,3-PCL) over poloxamer films (FLM-4-PCL) is due to phospholipids and a higher amount of chitosan. Although initial attempts to incorporate paclitaxel with phospholipids were found to be feasible, since unsaturated lipids are prone to oxidation, only chitosan-poloxamer films containing paclitaxel were chosen for further characterization unless specified.

Chemical Stability of Paclitaxel

In the present study, films were prepared by the classical method, which involves spreading a uniform layer of polymer dispersion followed by a drying step for removal of solvent system. Since film preparation methodology involved a heating step, it may have had a detrimental effect on the chemical stability of drug. Hence, stability assessment of paclitaxel impregnated in film was done using stability indicating method. For this purpose, paclitaxel was extracted from film and analyzed by HPLC. A single peak at 18.5 minutes representing paclitaxel (with no additional peaks) was detected in the chromatogram, suggesting that the molecule was stable during preparation of films (chromatograms not shown).

Content Uniformity

Paclitaxel was extracted from different regions of chitosan film using acetonitrile:ETOH (1:1 vol/vol) solvent system. After normalization of amount of paclitaxel on weight basis of film, the results indicated that the variation in distribution of paclitaxel in different regions of film was <15% (results not shown).

Release Studies

In order to establish the ability of films to serve as depot formulations, release of paclitaxel from both lipid- and poloxamer-containing films was studied. It was observed that release was negligible from lipid-containing films, while those containing poloxamer showed a burst effect followed by no release. Films containing poloxamer released ~10% in 6 hours; further release of paclitaxel was not observed until the study period of 144 hours, suggesting that the film had retained 90% of the payload (Figure 1A). Since the release of paclitaxel was <10%, further studies were undertaken in presence of lysozyme (chitosan is a substrate for the enzyme) to simulate the in vivo conditions. However, no significant difference was observed in presence of lysozyme (Figure 1B), suggesting that this model was not appropriate to simulate in vivo conditions for release-rate studies.

Mechanical Strength of Film

Mechanical strength of film is described in terms of tensile strength, and brittle films are characterized by a decrease in

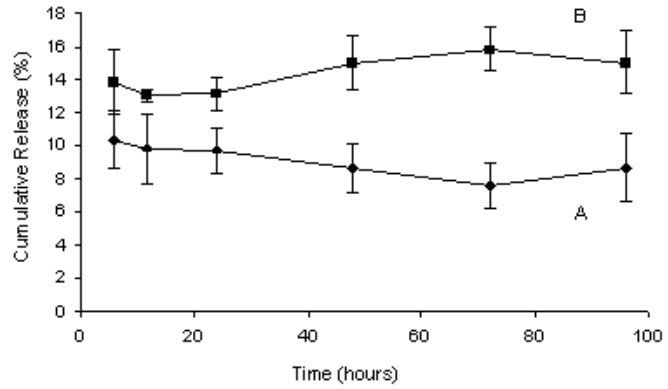

Figure 1. Cumulative percentage release (in vitro) of paclitaxel from chitosan film at 37°C in (A) absence and (B) presence of lysozyme (2 mg/100mL); 0.1% Tween 80 was used in release medium to provide sink conditions (data are mean ± SD; n = 3).

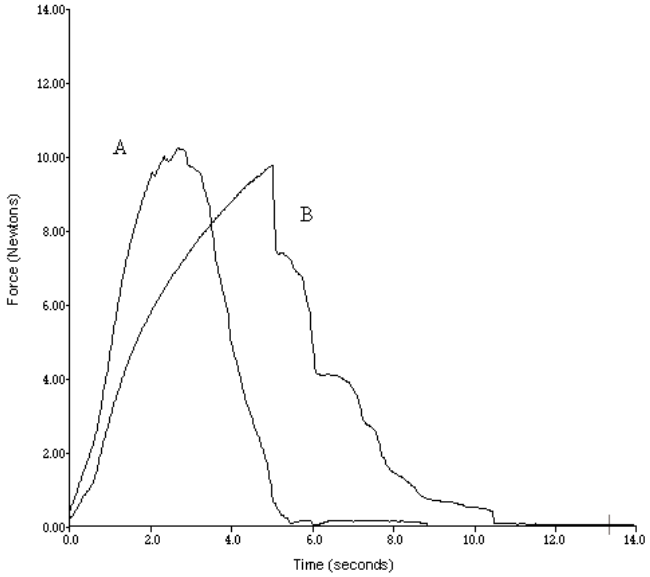

Figure 2. Force-time profiles of (A) blank film and (B) paclitaxel chitosan film to study the influence of formulation approach on mechanical properties of film.

the percentage of elongation at break. The area under curve is related to the energy required to break polymeric material, and tough polymers have larger areas requiring large amounts of energy for rupture. In order to understand the arrangement of polymer chains in the presence of paclitaxel, force-time profiles of films were generated as shown in Figure 2. Although force of elongation at break is slightly lowered in presence of paclitaxel in film (10.3 vs 9.8 N), the area under the profile has been increased (31.3 vs 45.9 N). For convenience of interpretation, each profile is further described in terms of ascending and descending portions. The time to plateau of the ascending portion of paclitaxel-chitosan film was greater in comparison with control film. As brittleness is reflected in the time to plateau, greater time is

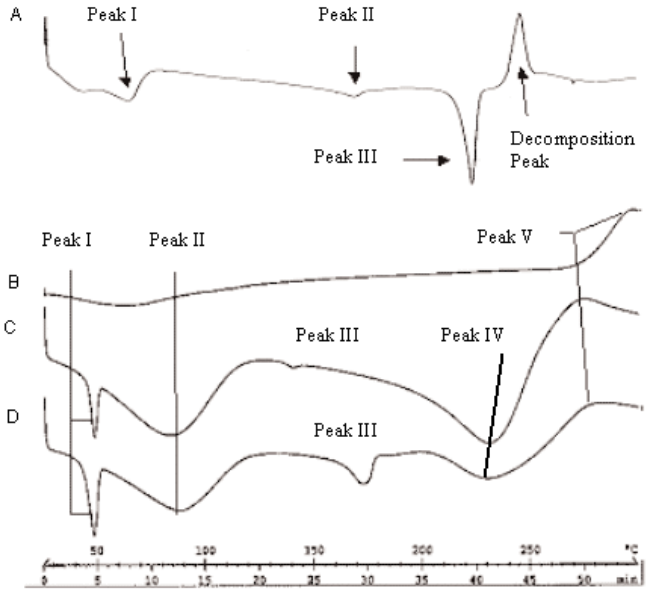

Figure 3. DSC studies to investigate physical transformations induced in chitosan and paclitaxel by comparing solid-state features of pure components with that in films (A) paclitaxel, (B) chitosan powder, (C) blank film, and (D) paclitaxel chitosan film. Thermograms were obtained at a scan rate of 5°C/min. When paclitaxel chitosan film was heated to 190°C, cooled to 25°C, and reheated, peaks I, II, and III were found to be irreversible in nature.

indicative of lack of brittleness of film. In addition, the descending segment of the profile of control film was uniform, while that of paclitaxel film was irregular and protracted. The discontinuities in internal structure and variation in strength of film matrix may be the cause of the irregular descending portion of the profile.

Solid-state Characterization

Thermal Studies of Films

The DSC thermograms of paclitaxel, recrystallized paclitaxel, blank, and paclitaxel-chitosan films are shown in Figure 3, and the observed thermal events are summarized in Table 2. Thermogram of paclitaxel showed an initial broad peak at 64.5°C (Peak I, Figure 3A) due to removal of absorbed moisture or nonstructural water followed by a single endotherm at 223.6°C (Peak III, Figure 3A) just prior to an exotherm of degradation peak. Another minor broad peak at 168.9°C (Peak II, Figure 3A) was observed, which is due to the presence of small amounts of paclitaxel dihydrate in the sample[19] (the peak was absent from second heating phase on DSC run when the sample was initially heated to 200°C, cooled back to 25°C, and reheated; results not shown). Liggins et al[19] have previously ascribed this peak to solid-solid transition associated with the conversion of dehydrated paclitaxel dihydrate to semicrystalline form. DSC studies were also performed on dry powder (recrystallized paclitaxel) obtained by evapora-

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.