Microencapsulated Controlled-Release Cisplatin Formulations for Oncology Applications

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ABSTRACT

Localized drug delivery technologies which enable sustained-release of the anti-cancer drug, Cisplatin, offer the potential for greater efficacy and reduced systemic toxicity. We report on a microencapsulation based formulation of Cisplatin, which enable controllable sustained-release of the active pharmaceutical ingredient (API). Furthermore, this technology has been developed into product concepts for multi-encapsulation of Cisplatin, as well as Radio Frequency Ablation device combined with drug delivery features.

Microencapsulation of Cisplatin was achieved with 75:25 poly-lactide-co-glycolide (PLGA) polymer. Microspheres were generated using a method employing solid-in-oil-in-water (S/O/W) emulsion with solvent evaporation, and were collected in 106-150 μm size ranges. The microspheres displayed uniform size and morphology, as illustrated by optical microscopy and SEM. Additionally consistent drug loadings for three distinct formulations in the range of 18-35% (w/w) was achieved, as measured by HPLC methodologies. Further characterization with pXRD and NMR established that during formulation, the API retains its cis-isoform (NMR) as well as its crystalline structure (pXRD). The in vitro release profile under ideal sink conditions yielded several days of sustained API release for the three distinct formulations. In contrast, non-encapsulated Cisplatin is completely released or dissolved in less than 4 hours. These controlled-releasing Cisplatin microsphere formulations are currently under evaluation in cell-culture to determine efficacy (i.e., IC-50 values) with 7 different cancer cell-lines, namely A549 (lung), 5637 (bladder), SKOV-3 (ovarian), HepG2 (liver), Caski (cervical), AsPC-1 (pancreatic and Tera-1 (testicular).

BACKGROUND

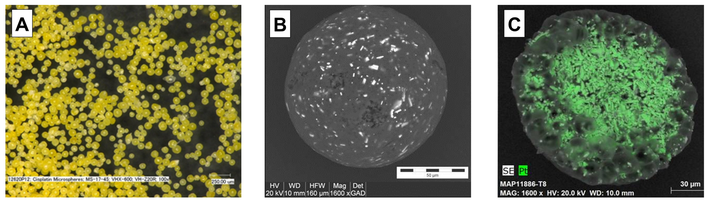
Cisplatin is widely used as an effective drug against a number of cancer diseases.[1] However, toxicity is a challenge, and this limits the dosage at which Cisplatin can be administered systemically.[2] In order to overcome this challenge, and to retain high drug efficacy at lower dosage, several attempts have been made in the past to modulate the release-profile kinetics of Cisplatin. For this purpose, Cisplatin formulations have been developed in the past based on microencapsulation,[3] liposomes,[4] gel-like matrices,[5] and other strategies.

Our goal was the development of polymeric microencapsulation based formulations of Cisplatin, with the specific goal of allowing for the use of the formulation with a variety of promising therapeutic strategies. In addition to the stand-alone use of the polymeric microencapsulation formulations for systemic administration, our goal includes (a) stand-alone local administration (b) local administration with the drug formulation incorporated into a medical device platform as a combination product, and (c) the above strategies in concert with other drug molecules (i.e., combination therapy administered both systemically and locally). The overarching motivation was to create a Cisplatin formulation with relevance to all the above diverse therapeutic strategies.

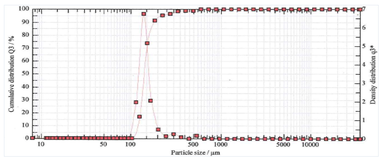
We have been successful in creating a range of microencapsulated Cisplatin formulations using poly-lactide-co-glycolide (PLGA) polymers, with the employment of solid in oil in water (s/o/w) emulsion based solvent evaporation methods. This has resulted in particles that are uniformly spherical and a wide particle size range - from the sub-micron (nanoparticle) range to 150 μm in diameter. Additionally, high drug loading levels were achieved, in the range of 30-35% (w/w), which is at the higher end for loading, compared to what is published in the literature.[6, 7] Most importantly, we have been successful in achieving a wide range of release-profile kinetics for Cisplatin, covering the range of release over a few hours to that over several weeks. The characterization of the formulation provides information on the morphology of the microencapsulated particles, the distribution of drug within them, as well as the crystalline state and isomer conformation of the drug molecule. These preliminary results provide the basis to optimize the performance characteristics of the formulation by a combination of variables such as dug-loading, type of PLGA polymer, molecular weight of encapsulating polymer, as well as process variables such as shear rate, speed of organic phase addition, and others. Preliminary data generated in cell culture with the A549 lung cancer cell line has established a significantly lower IC-50 value for the microencapsulated Cisplatin formulation compared to un-encapsulated Cisplatin, thus emphasizing the increased potency of our formulation with the promise of better efficacy at lower drug dosage.

In addition, we have been successful in achieving double-encapsulation / multi-encapsulation for Cisplatin, which opens up significant new possibilities, such as (a) co-encapsulation of two or more drugs, including Cisplatin and (b) multiple bursts in the kinetics of Cisplatin release profile. We have also been successful in crystallizing new combination product concepts, such as incorporating drug delivery components into RF Ablation devices through which the microencapsulated Cisplatin formulation could be administered locally into the tumor ablation margins.

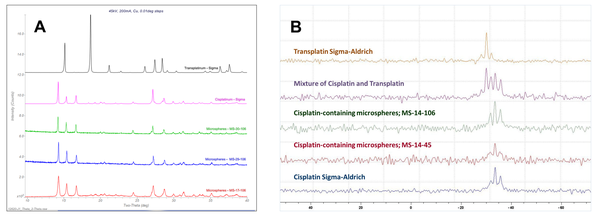
These preliminary results provide an encouraging basis for our ongoing work, namely further characterization and optimization of our formulation, followed by the evaluation of their efficacy in cell-culture and preclinical models.



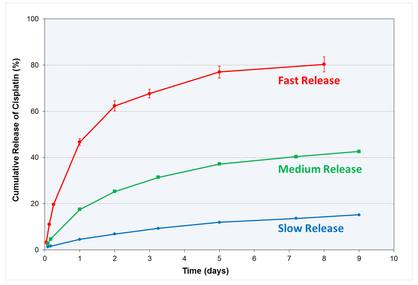
Images of 35% (w/w) Cisplatin loaded 75:25 PLGA microspheres (Fast Release). (A) Optical image (B) SEM image of one sphere. (C) SEM/EDS map of a cross-sectioned sphere showing a high concentration of Cisplatin.



Narrow particle-size distribution (Fast Release) shows the process is robust and capable.

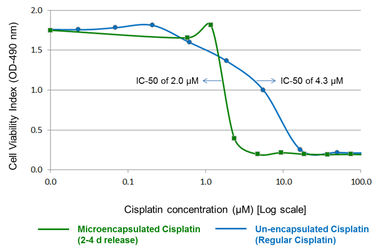


Cisplatin-containing microspheres characterized by (A) p-XRD and (B) natural abundance ^{15}N solid-state CPMAS (Cross Polarization Magic Angle Spinning) NMR. Cisplatin containing microspheres were found to compare well with the authentic sample of Cisplatin; and were clearly distinguishable from the like spectrum of an authentic Transplatin.

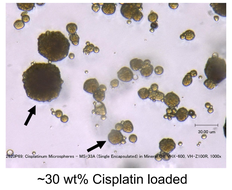


Modulated release of has been achieved in three different microsphere formulations. Error bars are based on standard error mean of n=3.

Preliminary Data: Inhibitory Concentration (IC-50) at 7 days was Reduced by Cisplatin Microspheres for the A549 Lung Cancer Cell Line



Novel Technology & Product Platform



~30 wt% Cisplatin loaded oxidized cellulose

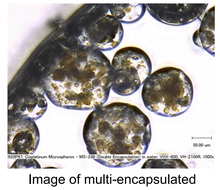
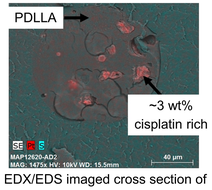
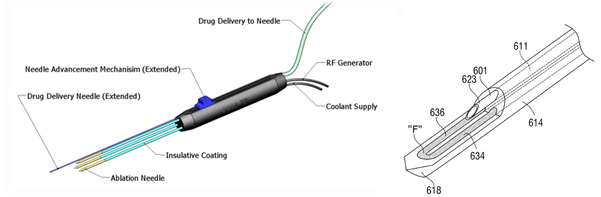


Image of multi-encapsulated microspheres



EDX/EDS imaged cross section of single multi encapsulated sphere

Integration of localized drug-delivery components into Cool-tip™ RF Ablation Device



CHARCTERIZATION METHODS

In-vitro release kinetics were measured by the application of experimental sink conditions (i.e., conditions allowing no more than 10% Cisplatin saturation). Approximately twenty-five milligrams of given batch of Cisplatin microspheres were added to dialysis tubing with a molecular weight cut off of 50,000 Daltons, and then submerged in phosphate buffered saline with Tween 20 at pH of 7.4 and maintained at 37°C. The elution of the Cisplatin from the dialysis tubing into the dissolution media was then measured by the collection of samples at each of the following time-points: 2, 4, 24, 48, 72, 120, 192 hours. The collected samples were subjected to HPLC analysis to determine Cisplatin concentration at different times, and ultimately as cumulative percent released vs. time. HPLC analysis was run on an Agilent 1200 LC system with a Waters μBondapak C18 10μm column using UV detection at 315nm.

Particle size distribution was obtained using Malvern Mastersizer 2000. SEM images were obtained utilizing an FEI Quanta 600FEG environmental scanning electron microscope. The powder x-ray diffraction (pXRD) patterns were collected using a Siemens/Bruker D500 diffractometer. NMR spectra were collected using Bruker Avance 700 equipped with a 4mm BB/^{1}H Cross Polarization Magic Angle Spinning (CPMAS) probe. A TL-2 triple resonance CPMAS probe was used to analyze the Transplatin sample.

The effect of Cisplatin formulations was evaluated in terms of cytotoxicity towards the A549 lung cancer cell line over a wide range of concentrations. The cells were plated in a 96-well plate at a density of 1,200 cells per well, between passage 2 and passage 8. Cell viability was evaluated at the day 7 time-point with the addition of Promega Substrate Cell Titer 96 Aqueous One Solution Reagent (tetrazolium compound), and absorbance reading at 490 nm. The Inhibitory Concentration (IC-50) was then determined for microencapsulated Cisplatin, as well as un-encapsulated, regular Cisplatin.

DISCUSSION

We have developed a range of Cisplatin formulations, which cover disparate Cisplatin release-profile kinetics ranging from release over a few hours to release over several weeks. Drug loadings ranging from 18-35 wt% was achieved, and at loadings significantly higher compared to previously published work. Further characterization methods demonstrated the robustness of our microencapsulation process because a narrow size fraction was achieved from 100-200 μm and the pXRD and NMR spectra suggest processing does not affect the cis -isoform and crystalline structure of Cisplatin. Preliminary cell-culture data has been encouraging, and form the basis for ongoing and planned cell culture and preclinical experimentation - with the specific purpose of optimizing the Cisplatin formulation for maximal efficacy. As we iteratively pursue cell culture and preclinical experimentation, we anticipate being able to achieve an optimized Cisplatin formulation with an adequate level of burst as well as slope (dy/dx) to address the drug bioavailability requirements. Additionally, we have developed a multi-encapsulation based Cisplatin formulation, with the promise of multi-drug release as well as multiple burst release involving Cisplatin. Localized delivery of Cisplatin formulations in conjugation with medical devices (e.g., RF Ablation devices) offers the promise of synergistic combinations of therapeutic strategies for pre-metastatic cancer treatment.

REFERENCES

1. Trimmer EE, Essigmann JM. *Cisplatin. Essays Biochem.* 1999; 34:191-211.
2. Gandara DR, Perez EA, Weibe V, De Gregorio MW. *Cisplatin chemo-protection and rescue: pharmacologic modulation of toxicity. Semin Oncol.* 1991 Feb; 18(1 Suppl 3): 49-55.
3. Lee YS, Lowe JP, Gilby E, Perera S, Rigby SP. *The initial release of Cisplatin from poly (lactide-co-glycolide) microspheres. Int. J Pharm.* 2010 Jan 4: 383(1-2):244-54.
4. Schroeder A, Honen R, Turjeman K, Gabizon A, Kost J, Barenholz Y. *Ultrasound triggered release of Cisplatin from liposomes in murine tumors. J Control Release.* 2009 Jul 1; 137(1): 63-8. Epub 2009 Mar 19.
5. *Cisplatin-conjugated porous gelatin particles: assessment of optimal conditions for binding and release. Cardiovasc. Intervent Radiol.* 2010 Aug; 33(4):813-7. Epub 2010 Jun 15.
6. Huo et al. *Studies on the poly(lactic-co-glycolic) acid microspheres of Cisplatin for lung-targeting. Int. J. of Pharmaceutics.* 2005. 289, 63-67.
7. Kyo et al. Effects of preparation conditions of Cisplatin-loaded microspheres on the in vitro release. *J. Control Release.* 1995. 35, 73-82.