CISPLATIN INCORPORATED PLGA NANOPARTICLES for LOCAL DRUG DELIVERY.

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Statement of Purpose: Maintaining a constant therapeutic level of a drug is problematic given the human body's ability to quickly clear foreign substances. Also some forms of drug therapy, such as chemotherapy, have a detrimental effect on the body with systemic release. The goal of our research is to utilize drug loaded nanoparticles to give a controlled extended and local drug therapy to alleviate both of these obstacles in the treatment of cancer. These nanoparticles are made from poly(D,L-lactic-coglycolic acid) (PLGA) that is both biodegradable and biocompatible.

Methods: Nanoparticles were prepared by using the basic principle of a double emulsion[1]. Cisplatin was dissolved in deionized water and added to a solution of (50:50) PLGA and dichloromethane (CH_2Cl_2). This mixture was then mixed using a probe sonicator. The resulting solution was then added to an aqueous solution of 1% polyvinyl alcohol (PVA), again mixed using the probe sonicator. The final solution was then placed under a hood overnight to remove the excess CH_2Cl_2. The next day the solution was filtered and then centrifuged. The pellet and supernatant were separated and then individually purified by further centrifuging. The remaining nanoparticles were dried overnight in a lyophilyzer.

Nanoparticles were then analyzed with light microscopy, and scanning electron microscopy (SEM) to determine their size and shape. Fourier transform infrared spectroscopy (FTIR) and x-ray diffraction crystallography (XRD) were used to determine cisplatin encapsulation. Experiments were also conducted with a sample of nanoparticles incubated for three weeks in 1X phosphate buffer solution (PBS) at 37°C while being constantly mixed on a stir plate. At regular intervals a sample was removed, centrifuged, separated and frozen. The supernatants of these samples were later thawed and then analyzed with UV spectroscopy at 301 nm to determine the amount of cisplatin that had been released compared with a standard curve. A similar technique was used to determine encapsulation efficiency where nanoparticles were first dissolved in CH_2Cl_2, and then PBS was added to the mixture. The released cisplatin would be dissolved in the PBS which would be separated from the CH_2Cl_2 and again analyzed with UV spectroscopy at 301 nm.

Results/Discussion: Light microscopy showed that submicron particles were formed, though not constituting the majority of the product (Figure 1). Some samples were filtered through a 0.8 μm filter to obtain a large percent of the submicron particles that were formed. FTIR and to a greater extent XRD showed that cisplatin encapsulation was successful. Peaks are shown in the cisplatin loaded nanoparticle's XRD spectra (Figure 2A) at roughly 27°, 38°, and 55° that coincide with peaks found in cisplatin's spectra (Figure 2B). Cisplatin release experiments showed a burst release profile for approximately three days with first a large release of cisplatin that then tapered down to a slower consistent release for the remainder of the experiment (Figure 3). After three weeks it is estimated that the nanoparticles had released approximately fifty percent of their cisplatin contents based on the encapsulation efficiency analysis that was done.

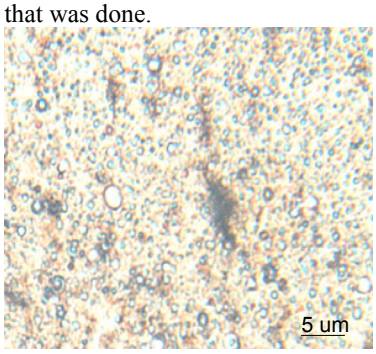

Figure 1. Light Microscope Image of Cisplatin Incorporated Nanoparticles.

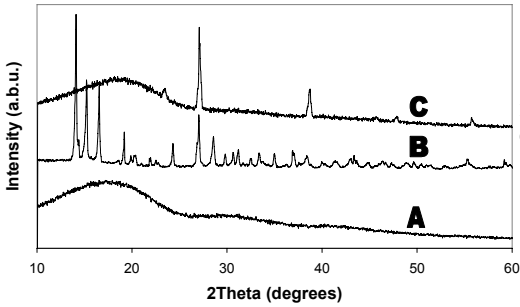

Figure 2. XRD Comparison of A) PLGA nanoparticles, B) Cisplatin, and C) Cisplatin loaded PLGA nanoparticles.

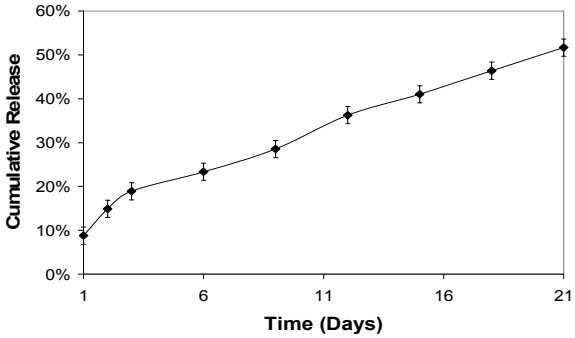

Figure 3. Cisplatin Release Profile.

Conclusions: An effective method has been established that produces approximately submicron particles from PLGA. The FTIR and XRD analyses shows that drug loading with cisplatin, is successful. UV analysis of incubation experiments shows a biphasic cisplatin release pattern. In future experiments the cisplatin loaded nanoparticles effectiveness as a drug therapy will be tested against cancer cell lines derived from osteosarcomas.

References:

1: Lamprecht, A. Int J of Pharm. 1999;184:97-105.