Microencapsulated Doxorubicin in PLA and PLGA Ultrasound Contrast Agents: Diagnosis and Therapy

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Introduction

Traditional forms of chemotherapy, in which highly toxic drugs are distributed evenly throughout the body, leads to systemic toxicity and organ failure. Due to the debilitating side effects, the dosage of chemotherapeutic is often restricted, lowering drug efficacy. This trade off has given rise to a broad area of research focusing on targeted drug delivery, increasing localized drug dosages, while minimizing systemic exposure and the associated side effects. Our area of focus has been the development of a platform for targeted, ultrasound-triggered drug delivery that functions both as a drug delivery vehicle, and also allows for medical imaging over the course of administration.

Ultrasound contrast agents (CA) have been selected as the primary drug delivery vehicle for a variety of reasons. Ultrasound is quickly emerging as one of the leading imaging modalities because it is safe, inexpensive, portable, and provides real time imaging. Ultrasound relies on the transmission of acoustic pressure waves through the body. Waves are partially reflected when encountering an impedance mismatch and this is used to create a digital image. The modality has been elevated in popularity due to the creation of higher resolution transducers, new imaging modalities, and numerous CA's (Lewin 2004). The possibility of CA was first noticed by Gramiak and Shah during an X-ray study of the aorta assisted by ultrasound. It was noticed that with the injection of indocyanine green, small air bubbles formed that produced a cloud on the ultrasound image (Gramiak 1968). Later it was determined that the acoustical impedance mismatch between the tissue and air caused almost perfect reflection of the acoustic wave, providing much better imaging contrast. In order to be functional, a CA must contain some form of impedance mismatch (usually gas), be stabilized with an outer shell to limit diffusion of the gas, be smaller than 8 microns in order to pass through pulmonary capillary bed, and durable enough to provide contrast over the duration of the ultrasound imaging (Narayan 1999).

Polymer contrast agents have been previously developed in our lab from mixtures of poly-lactic-co-glycolic-acid (PLGA) and pure poly lactide (PLA). These agents are fabricated using a double emulsion process, have a mean diameter of 1.2 μm, provide roughly 20 dB of ultrasound enhancement, and remain stable in the acoustic field for over 20 minutes (El-Sherif 2003). Effects of process parameters, polymer composition, molecular weight, and end capping have all been well documented. Current research efforts have focused on microencapsulation of doxorubicin (DOX). within these agents, allowing for localized delivery triggered by high pressured ultrasound waves. DOX is a well studied chemotherapeutic that interferes with topoisomerase II, preventing DNA replication (Momparler 1976). However, treatment of cancer with DOX has been linked with numerous side effects including congestive heart failure (Van Hoff 1979; Singal 1998; Ortho Biotech Products 2007). This tradeoff between potency and organ toxicity makes the drug an ideal case for targeted delivery using our CA platform.

Three methods of drug association within CA's have been developed and investigated within our laboratory. Incorporation involves the encapsulation of drug within the shell of the agent, dry adsorption relies on an electrostatic attachment of the drug to the surface of the agent after production, and wet adsorption relies on an electrostatic attachment of drug to the surface of the agent during production. Drug load and encapsulation efficiency have been optimized without compromising the acoustic properties or stability of the agent. Loading was investigated using a

50:50 PLGA blend and a pure PLA polymer. These results show feasibility for targeted drug delivery with ultrasound triggered release using polymer CA's.

Material and Methods

A double emulsion process was used to create both PLA and PLGA CA's (El-Sherif 2003). Briefly, 0.5 g of 50:50 PLGA and 0.05 g of camphor were dissolved in 10 ml of methylene chloride. 1 ml of 4% (w/v) ammonium carbonate solution was added and the mixture sonicated at 110 Watts for 30 seconds at 3 seconds on and 1 second off. The resulting (W/O) emulsion was then poured into 50 ml of 4°C, 5 % (w/v) polyvinyl alcohol solution (PVA) and homogenized for 5 min at 9000 rpm. 100 ml of 2% isopropanol solution was then added to the double emulsion and stirred for one hour to evaporate organic solvents. The microcapsules were then collected using centrifugation and washed three times with hexane. The capsules were then flash frozen and lyophilized for 48 hours. Camphor and ammonium carbonate sublime in the lypophiliser, leaving a void in their place that later fills with air after being exposed to atmospheric pressure. Samples are stored at -20^{o}C in a desicator until use.

As mentioned previously, different concentrations of DOX were added at different stages of the encapsulation, providing several drug loading techniques. For dry adsorption, 0.1g of dry polymeric microcapsules were suspended in PBS, containing 0.1-8 mg of DOX, for 0.5 hour, at room temperature, with agitation. The suspension was centrifuged for 5 minutes at 1,000 g. The microcapsules were then freeze dried for 48 hours. For wet adsorption, drug was added after hexane washing in varying concentrations from 0.5-40 mg w/w to create the desired loading percent. Samples were then washed and freeze dried. For incorporation, 0.1-4% w/w DOX was added to the primary mixture of PLA or PLGA in methylene chloride. The process of preparing the CA was then carried out as usual.
PLA and 50:50 PLGA were purchased from Lakeshore Biomaterials. PVA 88% mole hydrolyzed, with a MW of 25,000 was from Sigma-Aldrich. (1R)-(+)-camphor and DOX were from Sigma-Aldrich. Ammonium carbonate was purchased from J.T. Baker. All other chemicals were reagent grade from Fisher Scientific.

The acoustic testing setup is shown in figure 1 (Basude 1999). A 5 MHz, 12.7 mm, 50.88 mm spherically focused transducer was focused through an acoustically transparent window of a 50 ml sample holder placed in a 37 ºC water bath (18.6 MΩ-cm deionized water). A pulser/receiver was used to generate an acoustic wave with pulse repletion frequency of 100 Hz. Signals were amplified 40 dB and analyzed in Labview. Ultrasound enhancement relative to baseline and as a function of agent dose gave a measure of enhancement and as a function of time gave a measure of stability.

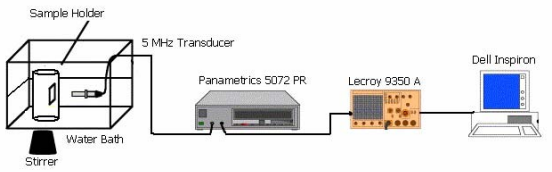


Figure 1: Acoustic setup used to measure ultrasound enhancement and stability of CA's.

Results

Varying concentrations of drug were successfully attached to both PLA and PLGA contrast agents using each of the three loading methods. Encapsulation efficiency was calculated for each concentration, loading method, and polymer and is shown below in Figure 2.

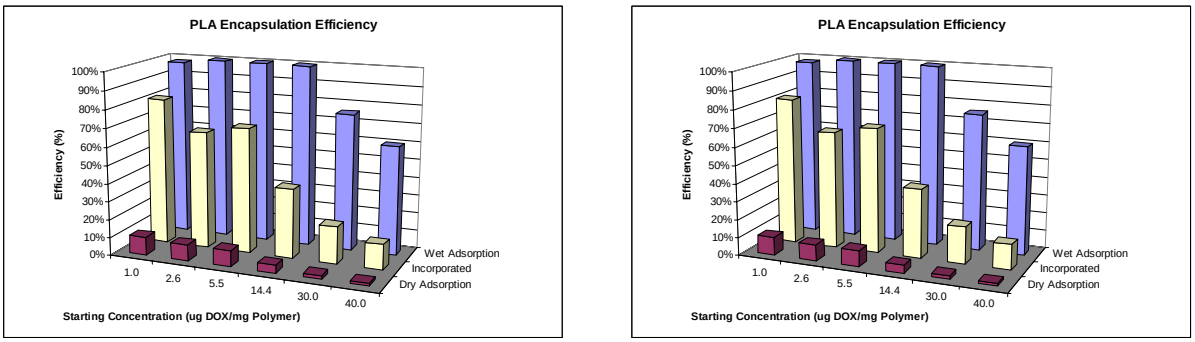


Figure 2: Encapsulation efficiency of DOX by concentration and loading method for PLA (Left) and PLGA (Right)

The effect of loading method on acoustic enhancement and agent stability was also studied. It was found that initial drug concentration did not have a statistically significant effect on either enhancement or stability (results not shown). However, the method of loading was seen to have an effect on these acoustic parameters. These results of enhancement by dosage for each method are shown in Figure 3.

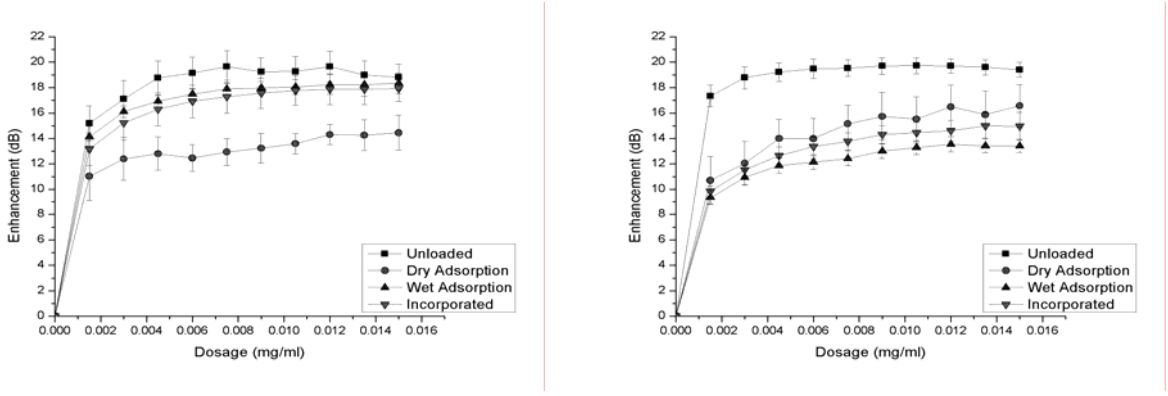


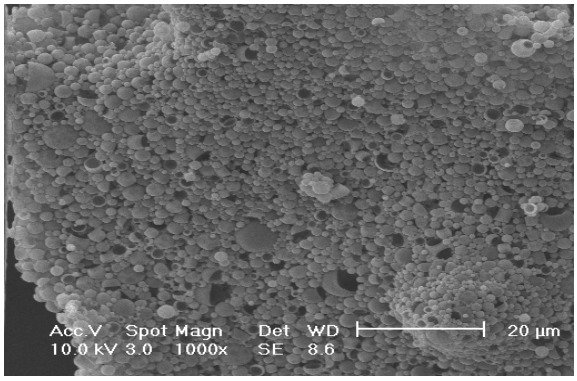
Figure 3: Ultrasound enhancement as a function of dose for each method for PLA (Left) and PLGA (Right)

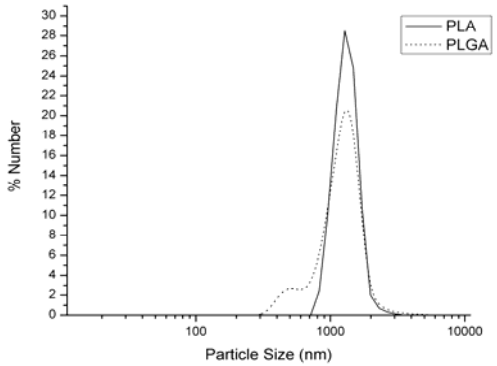
SEM Imaging and particle sizing were done on the capsules to ensure a smooth morphology, tight size distribution, and that all particles were less than the 8 µm size restrictions. Figures 4 and 5 show a representative SEM image and particle size distribution for PLA and PLGA samples. No trends in morphology or particle size were seen among loading methods or loading concentrations.

Discussion:

DOX was successfully loaded onto the CA's while still maintaining their acoustic properties. For each polymer, wet adsorption showed the highest loading efficiency, followed by incorporation, followed by dry adsorption. PLGA consistently showed higher encapsulation efficiency than PLA, possibly due to the polymer's less hydrophobic nature. PLA proved to be much more robust in the acoustic field. The PLGA agent was seen to show a much sharper loss of enhancement when drug was added using any method. This could be due to the polymer's lower glass transition temperature and less hydrophobic nature. Signal enhancement over time was also measured to ensure the contrast agents could be used for imaging during the duration of the ultrasound scan. PLA proved to be much more robust than PLGA, although both agents showed acoustic enhancement half-lives of well over 20 minutes (Results not shown). For both polymers, stability was only affected by the

loading of drug using the incorporation loading method. This may be due to the presence of drug within the polymer shell creating voids, making it more susceptible to hydrolysis. These results show a trade off between acoustic properties and drug payload. Particle size and surface morphology were consistent throughout the loading process. The retention of the smooth surface morphology was important for future tumor specific ligand attachments to the surface of the CA (Lathia 2004).





Figures 4-5: SEM image (Left) of a representative sample (Magnification= 1000X Size Bar=20 µm) and particle size distributions of a representative PLA and PLGA sample (Particle sizing done using dynamic light scattering).

Conclusions

Polymer ultrasound contrast agents have successfully been created with chemotherapeutics both in the shell and on the surface. These agents have retained enough of their acoustic properties to be used for both medical imaging and targeted, ultrasound-triggered drug delivery. Tradeoffs between drug payload and acoustic imaging are seen between polymer and loading methods, and may be optimized on a situational basis. Ultrasound parameters for optimal release are currently being investigated as well as alternative drugs and polymer parameters.

References

Basude, R. Wheatley, M.A. (1999) *Generation of ultrahamonics in surfactant based ultrasound contrast agents: use and advantages.* Ultrasonics 39, 437-444.
El-Sherif, D. Wheatley, M.A. (2003) *Development of a novel method for the synthesis of a polymer ultrasound contrast agent.* Journal of Biomedical Materials, 66A, 247-257.
Gramiak, R. Shah, P.M. (1968) *Echocardiography of the aortic root.* Invest Radiology 3, 356-366.
Lathia, J.D. et al. (2004) *Polymeric contrast agents with targeting potential*. Ultrasonics 42, 763-768.
Lewin, P. (2004) *Quo vadis medical ultrasound.* Ultrasonics 42, 1-7.
Momparler, R.L. et al. (1976) *Effect of adriamycin on DNA, RNA and Protein Synthesis in cell-free systems and intact cells.* Cancer Research 36, 2891-2895.
Narayan, P. Wheatley, M.A. (1999) *Preparation and characterization of hollow microcapsules for use as ultrasound contrast agents.* Polymer Engineering and Science 39, 2242-2255.
Orthod Biotech Products, L.P. (2007) *DOXIL Product Information.*
Singal, P. Ilskovic, N. (1998) *Doxorubicin-induced cardiomyophathy.* New England Journal of Medicine 339, 900-905.
Van Hoff (1979) *Risk factors of doxorubicin-induced congestive heart failure.* Annals of Internal Medicine 91, 710-717.