Polymersomes: Versatile Vesicles for Imaging and Drug Delivery

DH Levine[1], PP Ghoroghchian[2], J Freudenberg[3], G Zhang[3], G Li[4], KP Davis[5], FS Bates[5], MJ Therien[4], R Murali[3], and DA Hammer[1,2]

[1]Chemical and Biomolecular Engineering, [2]Bioengineering, [3]Pathology and Laboratory Medicine, [4]Chemistry, University of Pennsylvania, PA, USA; [5]Chemical Engineering, University of Minnesota.

Statement of Purpose: Polymersomes (polymer vesicles) have attractive biomaterial properties compared to phospholipid vesicles, including prolonged circulation times, increased mechanical stability, and the unique ability to incorporate hydrophobic molecules within their thick lamellar membranes and hydrophilic molecules within their core[1-4]. We can generate self-assembled nano-sized vesicles comprised of a biocompatible diblock copolymer consisting of polyethyleneoxide (PEO) and polybutadine (PBD) and a second comprised of fully-bioresorbable FDA-approved blocks: polyethyleneoxide (PEO) and polycaprolactone (PCL)[5]. In addition, we have successfully loaded imaging agents, such as porphyrin based near infrared (NIR) fluorophores[6-9], and therapeutics such as doxorubicin, an anti-neoplastic agent[5], into these polymersomes and tracked their release *in situ* and *in vivo*. This study highlights the enormous promise for using polymersomes as *in vivo* imaging and drug delivery agents.

Methods: Thin-film hydration was used to assemble the PEO-b-PCL copolymers into equilibrium morphologies [5]. Polymersomes were incubated with doxorubicin in a ratio of 1:.4 polymer:drug (w/w) for ~9h at a temperature above their main gel to liquid-crystalline phase transition temperature, trapping the drug in the aqueous core. Nonentraped DOX was removed using HPLC; the solution was passed through two desalting columns and further removed using a Centricon tube to ensure the absorbance of the subnatant was undetectable at 480nm. The collected DOX polymersome solution was concentrated and passed through a 1um membrane prior to injection. Sample aliquots were removed and lyophilized to destroy the vesicle structure and release the encapsulated DOX. The freeze-dried powder was reconstituted, and the DOX concentration was determined by measuring the absorbance at 480nm.

In vivo tumor studies: T6-17 tumor cells ($1x10^6$) were injected *s.c.* into the flank of athymic nude female mice. After seven days, the mice were injected through the tail vein with 200uL of (1) DOX polymersomes, (2) DOXIL (liposomal formulation of DOX), (3) free DOX, and (4) PBS. The concentration of DOX was 1mg/ml. Tumor size was measured daily.

IN VIVO PORPHYRIN BIODISTRIBUTION STUDY: Preparation of vesicles: Self-assembly via thin-film hydration followed by freeze thawing and extrusion were used to yield small porphyrin PEO-b-PBD polymer vesicles (<300nm diameter)[7]. The porphyrin dye is localized to the vesicle membrane. The suspension was centrifuged to obtain a porphyrin concentration of 15uM as determined by absorbance spectroscopy.

In vivo biodistribution: 100ul of the porphryin polymersome solution (15uM porphyrin) was injected intravenously into the tail vein of a tumor bearing (T6-17 cells) nude mouse. Fluorescent signal was measured prior to injection as well as at specific time points post injection.

Results: Porphyrin polymersomes injected into the tail vein of a tumor bearing mouse accumulated at the tumor site of non-necrotic tumors within four hours, and were cleared by organs of the RES as determined by imaging of fluorescence signals. Furthermore, we administered polymersomes *in vivo* and assessed the therapeutic capability of drug loaded polymersomes in retarding tumor growth. Figure 1 shows doxorubicin loaded polymersomes retarded tumor growth in a live animal on par with the commercially available DOXIL.

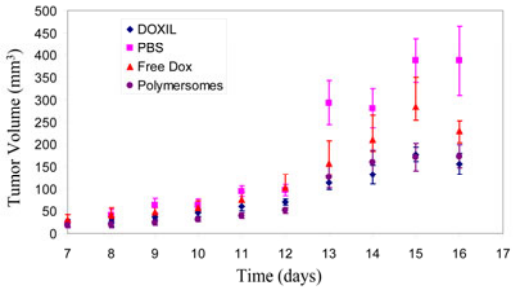

Figure 1. The Effect of Treatments on Tumor Volume Over Time. Bars=SEM, n=5.

Conclusions: We have shown that porphyrin PEO-b-PBD polymersomes can be used to non-invasively track the location of polymersomes, and may potentially be applied for diagnostic studies. Porphyrin polymersomes will greatly decrease the number of animals required for biodistribution since the location of the polymersomes can be determined without sacrificing animals at multiple time points to perform histology on the excised organs. Furthermore, we demonstrated the ability of drug loaded PEO-b-PCL polymersomes to retard tumor growth. The ability to load components into the membrane and core shows enormous promise for future dual modality polymersomes that enable both imaging and drug delivery. Hence, polymersomes have potential to be nanostructured biomaterials for future drug delivery and imaging applications.

References:

([1]Bermudez H. Macromolecules. 2002;35:8203-8208.)
([2]Lee JCM. Biotechnol. Bioeng. 2001;73:135-145.)
([3]Meng FJ. Controlled Release. 2005;101:187-198.)
([4]Photos PJ. J. Controlled Release. 2003;90:323-334.)
([5]Ghoroghchian PP. Macromolecules. 2006;39:1673-1675.)
([6]Ghoroghchian PP. Chem. Mater. 2007;19:1309-1318.)
([7]Ghoroghchian PP. PNAS USA. 2005102:2922-2927.)
([8]Ghoroghchian PP. JACS. 2005;127:15388-15390.)
([9]Ghoroghchian PP. Soft Matter. 2006;2:973-980.)