Release and Uptake of Pluronic from *In Situ* Forming PLGA Implants

Angela Carlson[1], Tianyi Krupka[2], Luis Solorio[1], and Agata Exner[1, 2]
Depts. of Biomedical Engineering[1] and Radiology[2], Case Western Reserve University, Cleveland, OH 44106

Statement of Purpose: Conventional drug delivery systems, either local or systemic, often deliver an ineffective dose of chemotherapeutic agent to the target tumor tissue which can lead to the development of drug resistance [1]. Our approach uses minimally invasive, *in situ* forming implants which consist of Poly(lactic-co-glycolic acid) a chemotherapeutic drug and Pluronic (PEO-PPO-PEO), an amphiphillic triblock polymer that has been added to modulate drug release [2] and more importantly, to act as a chemosensitizer [3]. This delivery system is advantageous because the chemosensitizer, Pluronic, reduces the drug dose required to stimulate a chemotherapeutic effect which prevents systemic toxicity and minimizes the development of drug resistance. In addition, this system is injectable and forms a roughly spherical implant *in situ* via phase-inversion when placed in a hydrophilic environment. The goal of this study was to characterize the effect of Pluronic structure on its release from degradable PLGA implants *in vitro* and its uptake by cancer cells.

Methods: Four Pluronics (MW: 2000-4600) were labeled with 5-(and-6) carboxy 2',7'-dichloro-fluorescein. Pluronic release was quantified by creating *in situ* forming implants comprised of PLGA and Pluronic dissolved in NMP that were injected into PBS, a hydrophilic environment. Samples of the PBS were taken at 0, 0.16 , 0.5, 1, 4, 8, 24, 48, 96 and 144 hrs and were analyzed for the presence of fluorescent Pluronic. Cell uptake was determined by treating rat colorectal carcinoma cells with Pluronic/media solutions (0-30mg/mL) for 0-6 hours. Excess Pluronic solution was washed away and the cells were analyzed for fluorescent Pluronic uptake.

Results: All Pluronics showed burst release in the first 8 hours followed by constant release until day 6 (Fig 1A). Pluronic L81 (MW 2750) showed the fastest release throughout the 2-6 day time points (Table 1). Pluronic release was also analyzed during the burst release phase and a linear correlation between the extent of release after 1 hr and the hydrophilic-lipophilic balance (HLB) of each Pluronic was observed (R^2=0.96). The cellular uptake of Pluronic was compared at the 1mg/mL dose. Pluronic L81 was taken up by the cells at a significantly higher concentration compared to the other analyzed Pluronics (Fig 1B, Table 1). A nonlinear correlation between the cellular uptake and the HLB of each Pluronic was observed (R^2=0.87) after treatment at 1mg/mL for 1 hr.

Pluronic	% Release (6 days)	Cellular Uptake at 1mg/mL and 1hr (µg)
L61	58.13±8.05	0.013±0.015
L62	53.07±0.49	0.008±0.009
L81	72.61±4.34	0.043±0.008
P85	53.15±4.19	0.004±0.011

Table 1 Pluronic release after 6 days and cellular uptake after 1 hr

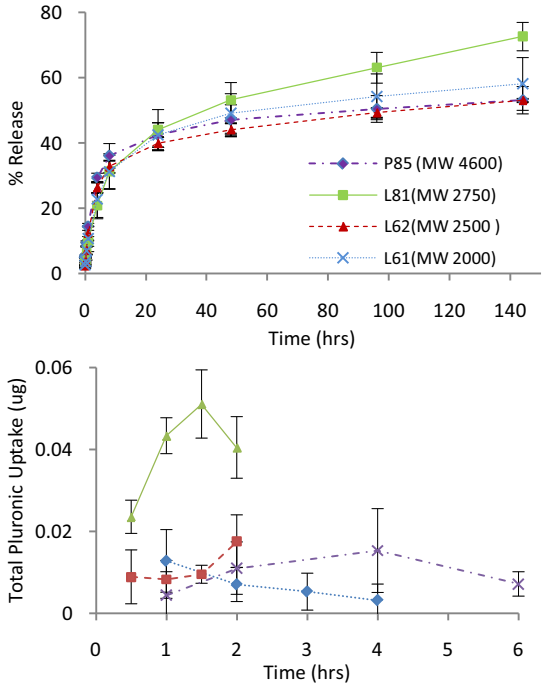

Fig 1 (A) Release of L61, L62, L81, and P85 from 0-6 days. (B) Cellular uptake of 1mg/mL of Pluronic after various exposure times. Data shown as mean ± SEM (standard error of mean) (n=4).

Conclusions: These preliminary results demonstrate the feasibility of incorporating Pluronic into PLGA implants. The release study showed that sufficient, extended release of Pluronic from PLGA implants occurred *in vitro*. In the uptake study, a dependence on treatment time, Pluronic dose as well as structural characteristics of the Pluronic was observed. L81 shows the most promise as a chemosensitizing candidate due to its increased release and uptake when compared with the other Pluronics studied. Future studies will determine the chemosensitzing efficacy of Pluronic when combined with a chemotherapeutic agent. This work was supported by R01CA118399 to AAE.

References:

1.Batrakova, E.V., Pharm Res. 2003; 10:1581-90
2.Patel, R.B.,J Biomed Mater Res A.2010; 94:476-84
3.Krupka,T.M., Exp Biol Med. 2009;234:95-104