Characterization of *in vivo* drug release from *in situ* forming drug delivery implants

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Statement of Purpose: Burst drug release from in situ forming drug delivery implants (ISFIs) has been shown to be highly dependent on implant phase inversion[1]. Recently, a study by our group has shown that polymer molecular weight (Mw) can play a role in controlling *in* vitro burst drug release of low Mw drugs from poly(D,L-lactide-co-glycolide) (PLGA) ISFIs[1]. However due to the significant effect that the external environment has on ISFI phase inversion, drug release from these systems may be quite different *in vivo* as well as in different tissue environments. In the current study, we examined the effect of varying Mw PLGA on ISFI drug release in an *in vivo* subcutaneous (SubQ) environment. Additionally, to determine the effect different types of tissue environments have on drug release from ISFIs, release into tumor tissue was investigated.

Methods: ISFI formulations were comprised of PLGA (Lakeshore Biomaterials, Birmingham, AL), Pluronic P85 (P85, Mw: 4600 Da), donated by BASF Corp. (Shreveport, LA), and 1-methyl-2-pyrrolidinone (NMP) from Sigma (St. Louis, MO). Fluorescein (376 Da) was used as a mock drug molecule that was dissolved in our implant solution. To study the effect of PLGA Mw on drug release, 50:50 PLGA 2A, 3A, and 4A with mean Mw of 18, 33, and 50 kDa, respectively, were varied in our different prepared formulations.

In vivo SubQ drug release was examined by injecting 0.05 mL of each polymer formulation (n=5) into the dorsal SubQ region of BD-IX rats. Animals were then sacrificed, and implants were removed at the following time points 1 hour, 1 day, 2 days, 4 days, and 7 days post implantation. Removed implants were degraded in a base solution, neutralized with acid, diluted in buffer (PBS, 7.4), and then analyzed using a fluorescence plate reader (Tecan Ltd., Infinite 200 series) with an Ex/Em wavelength of 485/535 nm. Mass of drug present in the degraded implant solution was obtained by comparison to a standard curve, and percent drug release was calculated using the initial drug loading mass for each implant.

To investigate drug release into tumor tissue, DHD/K12/TRb rat colorectal carcinoma cells were inoculated into BD-IX rats and tumors were grown to a mean diameter of 1-1.5 cm using previously established methods[2]. 0.05 mL of the PLGA 3A formulation was then injected into the tumor tissue, and drug release was examined using the process described above. All animal studies and protocols were IACUC approved.

Results/Discussion: The drug release data from varying Mw PLGA formulations indicates a trend whereby high Mw PLGA formulations have a decreased burst drug release in comparison to low Mw formulations (Fig 1A). While these results are supported by previous *in vivo* drug release results[3], the trend is opposite of what was previously seen *in vitro*[1]. Additionally, *in vivo* burst drug release, 37.2%, measured 1 hour after implant injection was significantly greater ($p<0.05$) than in an *in vitro* environment, 16.7% (Fig 1B). When ISFIs were injected into tumors, a similar trend was seen with intratumoral burst drug release from ISFIs being much greater than *in vitro.* However, since the tumor model had a high degree of variability within each lesion, with approximately half the tumors being necrotic and the other half non-necrotic, a different drug release profile was found between the two tumor types. Release into necrotic tumors followed the same profile seen in a SubQ environment, while implants placed in non-necrotic tumors release drug much faster (Fig. 1B).

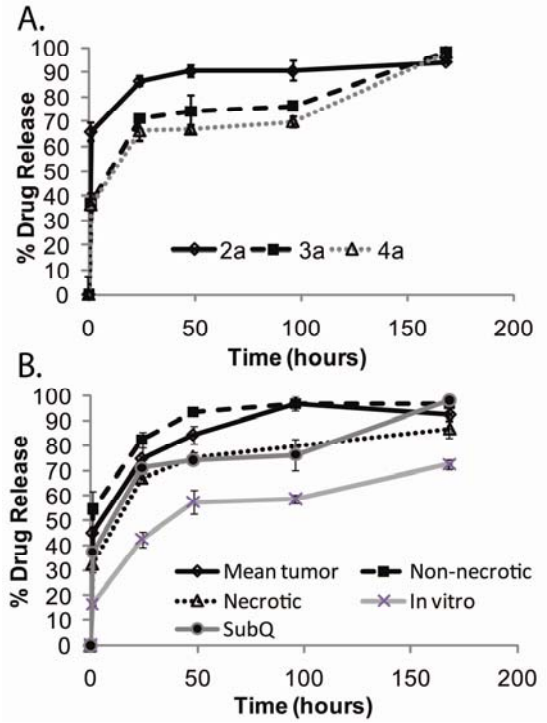

Figure 1. **(A)** Percent of initial drug mass released as a function of time is shown for varying Mw PLGA formulations in an *in vivo* SubQ environment. **(B)** Percent drug release from ISFIs in a variety of environments including in necrotic tumor tissue, non-necrotic tumor, SubQ tissue, and *in vitro* bath solutions is shown. Mean tumor curve averages all tumor trials.

Conclusions: Our study shows that drug release from ISFIs is highly dependent on the external tissue environment in which they are placed. Additionally, there seems to be poor correlation between implant behavior *in vitro* and *in vivo.* This work was supported by R01CA118399 to AAE and T32GM07250.

References:

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