Cyclodextrin Enhances In Situ Forming Implant Affinity with Drug and Controls Drug Release

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Statement of Purpose: Poly(lactic-co-glycolid acid) (PLGA)-based *in situ* forming implants (ISFI) are biocompatible and biodegradable injectable delivery systems which form a solid drug-eluting depot at the injection site. Because the therapy only requires minimally invasive injection and the release can continue over weeks to months, it has been investigated for local long-term cancer treatment.[1] Previous work in our lab has shown that the addition of cyclodextrin (CD) to non-refillable polymers allows for substantial drug refilling and additional windows of drug delivery.[2] We decided to explore the effects of CD incorporation on ISFI behavior, as well as how the molecular weight and monomer ratio of PLGA affects drug refilling. We hypothesized that incorporating CD, increasing PLGA hydrophobicity, and decreasing PLGA molecular weight will increase drug refilling into ISFIs.

Methods: The following copolymers were purchased from Akina, Inc. (West Lafayette, IN): PLGA L:G (lactide: glycolide) 50:50 and L:G 75:25 of molecular weight (MW) 15-25 kDa, 35-45 kDa; PLGA L:G 100:0/Poly(D,L-lactic acid) (PDLLA) at MW 15-25 kDa, 25-35 kDa. Insoluble CD particles were synthesized as previously described.[2] Briefly, soluble CD prepolymer (CycloLab, Budapest, Hungary) was reacted with ethylene glycol diglycidyl ether cross-linker (Polysciences Inc, Warrington, PA) in 0.2M potassium hydroxide for 2 hours at 60°C in light mineral oil with 1% surfactant to emulsify. Particles were washed, frozen, and finally lyophilized. The formation of ISFIs was described by Solorio et al.[1] Briefly, polymer solutions were made with 6:4 mass ratio of n-methyl-2-pyrrolidinone (NMP, Fisher, Hampton, NH) to polymer (or 6:3:1 NMP:polymer:CD particles), pipetted into phosphate-buffered saline (PBS) to start formation, and then weighed. Implants were stored at 37C and 100rpm for at least 7 days prior to use. Setup of agarose refilling assays was described by Cyphert et al[2]. Briefly, implants (n=9) were embedded in agarose gels in 6-well plates with 2mg/mL doxorubicin (DOX, AChemblocks, San Francisco, CA) in water in the center of each well. Plates were stored at 37°C and 100 rpm for 3 days to facilitate refilling. Refilled implants were dissolved in dimethylsulfoxide (DMSO) and then were analyzed via absorbance and fluorescence spectroscopy on a Biotek Synergy H1 microplate reader (Winooski, VT). Refilled drug was quantified by comparing spectroscopy data to standard curves. Refilled drug mas was divided by the final polymer mass and reported as normalized drug/polymer mass. Statistical testing was performed in Prism GraphPad (San Diego, CA) using One-way ANOVA.

Results: The incorporation of CD polymers resulted in at least a 2-fold increase in refilled drug, with one exception at PDLLA 15-25 kDa. PLGA 50:50 L:G with MW 15-25kDa with CD has a 2.2-fold increase in refilling over the same polymer implants without CD (p=0.003). PLGA 50:50 L:G at 35-45 kDa has 2.7-fold increase (p<0.0001). PLGA 75:25 L:G at 15-25 kDa with CD has 2.04-fold increase (p=0.0006). PLGA 75:25 L:G at 35-45 kDa with CD has 8.04-fold increase (p=0.0037). PDLLA at 15-25 kDa with CD has non-significant 1.27-fold increase (p=0.1381). PDLLA at 25-35 kDa has 3-fold increase (p=0.0031). Overall, PLGA 50:50 L:G at the lowest MW used of 15-25kDa with CD incorporated experienced the highest level of refilling compared to any of the other groups (**Fig. 1A**). Increasing MW does not consistently increase drug refilling, as seen with non-significant differences in refilling between 15-25 kDa and 35-45 kDa without CD PLGA 50:50 L:G (**Fig. 1A**), between 15-25 kDa and 25-35 kDa without and with CD PDLLA (**Fig. 1C**). Increasing L:G monomer ratio, and hydrophobicity of the implants, consistently resulted in decreased refilling when going from 50:50 to 100:0 (PDLLA) L:G (p<0.0001 **Fig. 1E-G**).

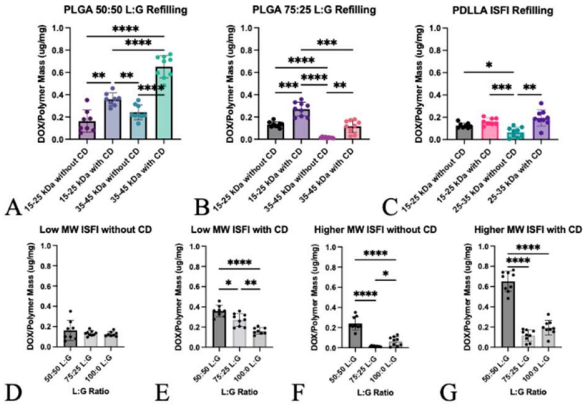

Figure 1. Drug refilling into **A**) PLGA 50:50 L:G, **B**) PLGA 75:25 L:G, and **C**) PLGA 100:0 L:G (PDLLA). Data in **A-C** replotted in **D-G**, re-depicted as refilling vs L:G monomer ratio, and separated into **D**) Low MW ISFI (15-25 kDa) without CD, **E**) Low MW ISFI with CD, **F**) Higher MW ISFI (25-35 and 35-45 kDa) without CD, and **G**) Higher MW ISFI with CD. Error bars depict standard deviation. *p<0.05. **p<0.01. ***p<0.001. ****p<0.0001.

Conclusion: Overall, drug refilling through agarose can be described as follows: 1) Incorporating CD significantly improved the amount of drug refilling. 2) Increasing PLGA MW did not show consistent trends, showing both increases and decreases in drug refilling. 3) Increasing the L:G monomer ratio generally decreased drug refilling. Initial drug release profiles are currently being assessed (data not shown). Future work will involve evaluating the drug release profiles of ISFI after being refilled as well as characterizing physical characteristics of ISFIs including inversion rate, swelling ratio, and degradation.

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References: [1] Solorio L. Ther Deliv. 2016;7(4):201-212. [2] Cyphert EL. Adv Healthcare Mater.2018;7:1800812.