Polymeric Microparticles for Controlled Fibrolysis in Abdominal Aortic Aneurysms (AAAs)

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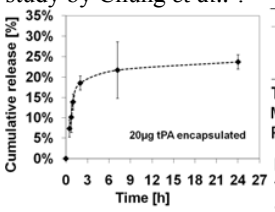
Statement of Purpose: AAAs are a major cause of death in the US. They are characterized by proteolysis of the aortic wall, by matrix metalloproteases (MMPs) -2 & -9. An intraluminal mural thrombus (ILT) is commonly present in AAAs[1], through which blood continues to flow, and plays a critical role in AAA progression via protease storage, release and activation[2]. The luminal surface of the ILT also undergoes constant renewal due to its contact with circulating blood components, leading to release of procoagulant materials & platelet aggregation. However, the abluminal surface undergoes fibrinolysis due to conversion of fibrin-bound plasminogen in the thrombus to plasmin by plasminogen activators in the adjacent AAA wall. The diverse pathways of thrombosis or coagulation mediated via thrombin, and thrombo- or fibrinolysis via plasmin(ogen) thus have been found to activate MMP-2, potentially forming a positive-feedback loop for AAA evolution[3,4]. Hence, there is a need for controlled delivery of fibrinolytic agents for highly modulated clot lysis, while avoiding systemic side-effects and potential deleterious effects of these agents in AAAs. We have developed & characterized PLGA microparticles (MPs) encapsulating tissue plasminogen activator (tPA), and examined their *in vitro* efficacy in lysing fibrin clots. Further, we propose to examine the effects of clot lysis products on MMP-synthesis & activity in culture with rat AAA smooth muscle cells (EaRASMC), as well as their thrombolytic efficacy *in vivo* in rat AAAs.

Methods: tPA-encapsulated PLGA MPs (50:50 lactide: glycolide) were formulated via a double emulsion solvent evaporation technique with polyvinyl alcohol (PVA) or didodecyldimethyl ammonium bromide (DMAB) as the stabilizer. PVA is widely used in MP formulation, and imparts them with a strong negative charge, while DMAB provides MPs with a strong positive charge[5]. Cationic MPs may exhibit improved clot targeting via electrostatic interactions, as fibrin is negatively-charged[6] at pH 7.4.

Size & surface charge (ζ-potential) of MPs was determined via phase analysis light scattering. tPA was conjugated with AlexaFluor 633 to enable its fluorometric detection in release studies carried out in PBS from 10 mg/mL MP suspensions. The ability of PVA- & DMAB-functionalized MPs (50 µg encapsulated tPA) to lyse fibrin clots was evaluated in preliminary studies. Clot lysis time defined as the time required for clot absorbance (at 405 nm) to decrease to 50% of its initial value[7].

Future studies will involve a more comprehensive comparison of in vitro clot lysis efficacy of PVA- & DMAB- functionalized MPs as a function of tPA loading & MP concentration. In parallel we will examine the effects of products released following clot lysis in a cell-culture insert on cell proliferation & MMP-production and activity by cultured EaRASMCs in well-plates. Optimal MPs would be expected to be those which demonstrate minimal clot lysis time and/or lowest tPA loading & release required for clot lysis, as well as decreased MMP activity in cell culture. Finally, we will examine the thrombolytic efficacy of the optimized dose of tPA-encapsulated MPs *in vivo* for clots created at rat AAA sites by concurrent infusion of elastase & plasmin. Microscopy will be used to examine clot architecture, as well the ability of MPs to permeate through micron-size pores in clots[8], and into the AAA wall.

Results: PVA-functionalized MPs encapsulating tPA had a mean size of ~900 nm, with a surface charge of -35 mV (Table 1), while DMAB-functionalized MPs showed a mean size of 1100 nm, with a surface charge of +30 mV. tPA was undetectable fluorometrically in the supernatant solution obtained during formulation, suggesting that the encapsulation efficiency was likely > 90%. tPA release was found to be plateuaued at ~25% over the first 24h of release (Fig.1), which is similar to those obtained in a study by Chung *et al.*.[6].



Sample	Size [nm]	ζ-Potential [mV]
Blank (tPA-free)	859.1 ± 28.5	-35.7 ± 4.2
20 µg tPA	904.6 ± 1.4	-33.3 ± 3.9
50 µg tPA	905.3 ± 0.5	-36.7 ± 4.5

Table 1. Size and surface charge PLGA MPs encapsulating tPA, formulated with PVA as stabilizer (mean ± SD, *n*=3/case)

Figure 1 (left). Release profile of tPA from PLGA MPs (10 mg/mL MPs; mean ± SD, *n*=3/case)

Although MPs were not as effective as the exogenous tPA in terms of clot lysis time (Fig.2), based on the release curves, we would expect the amount of tPA released to be lower than the exogenous dose. DMAB-based MPs exhibited slower fibrinolysis compared to PVA-based MPs, potentially due to their electrostatic interactions with the fibrin in the clot. Slower fibrinolysis may be desirable from the standpoint of decreasing plasmin release post-lysis, potentially leading to reduced MMP synthesis and activity *in vitro*.

The planned *in vitro* & *in vivo* studies will provide deeper insights into the extent of fibrinolysis & NP formulation/dose desirable from the standpoint of AAAs, to avoid MMP-activation & subsequent progression of AAAs. Overall, these studies will help develop a suitable MP-based paradigm for regulating the AAA environment for subsequent delivery of AAA therapy.

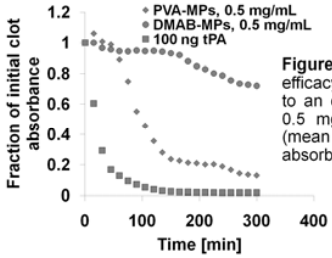


Figure 2. Comparison of fibrinolytic efficacy of MPs loaded with 50 µg tPA to an exogenous dose of 100 ng tPA. 0.5 mg/mL MPs contain 208 ng tPA (mean values shown for *n*=3/case; clot absorbance measured every 15 min)

References: 1. Adolph *et al.*. J Vasc Surg 1997; 25:916-26. **2.** Fontaine *et al.*. Am J Pathol 2002; 161:1701-10. **3.** Galis *et al.*. Circ Res 2002; 90:251-62. **4.** Carmeliet *et al.*. Nat Genet 1997; 17:439-44. **5**. Labhasetwar *et al.*. J Pharm Sci. 1998;87:1229-34. **6.** Chung *et al.*. Biomaterials 2008; 29: 228-237. **7.** Undas *et al.*. Blood. 2009; 114: 4272-8. **8.** Janmey *et al.*. Blood 1992; 80: 928-36.