Controlled Fibrinolysis via Localized Nanotherapeutic Delivery in Abdominal Aortic Aneurysms (AAAs)

Balakrishnan Sivaraman[1], Andrew Sylvester[1,2], Anand Ramamurthi[1].

Department of Biomedical Engineering, Cleveland Clinic [1] and Case Western Reserve University[2], Cleveland OH.

Statement of Purpose: AAAs are the 13th leading 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 present in ~75% of AAAs[1], through which blood continues to flow. It plays a critical role in AAA progression via storage, release & activation of proteases & inflammatory cells[2], which degrade elastin/elastic matrix, potentially leading to AAA progression[3,4]. Hence, there is a need for highly modulated clot lysis, while avoiding potential deleterious effects lysis products on AAAs. We have developed & characterized PLGA nanoparticles (NPs) encapsulating tissue plasminogen activator (tPA;fibrinolytic drug), and examined their *in vitro* efficacy in lysing fibrin clots. Further, we examined effects of clot lysis products on elastic matrix deposition, MMP-synthesis & activity in rat AAA smooth muscle cell (EaRASMC) cultures.

Methods: tPA-encapsulated PLGA NPs were formulated via a double emulsion solvent evaporation technique with polyvinyl alcohol (PVA) or didodecyldimethyl ammonium bromide (DMAB) as the stabilizer. PVA & DMAB impart NPs with a negative and positive surface charge, respectively[5,6]. We have shown cationic NPs to exhibit improved elastin binding, elastic matrix deposition and MMP-inhibition in vitro in EaRASMC cultures[6], illustrating their benefits from a AAA standpoint.

Size & surface charge (ζ-potential) of NPs was determined via phase analysis light scattering. tPA was conjugated with AlexaFluor 633 to enable its fluorometric detection in release (10 mg/mL NPs; PBS) & clot-binding studies (0.2, 0.5, 1.0 mg/mL NPs). The ability of PVA- & DMAB-functionalized NPs (10, 20, and 50 µg encapsulated tPA) to lyse fibrin clots was evaluated. Clot lysis time is defined as time required for clot absorbance (at 405 nm) to decrease to 50% of its initial value[8]. Elastic matrix (Fastin assay) & MMP outcomes (gel zymography, western blot) in EaRASMC cultures following clot lysis in a transwell assay were also evaluated for 10µg tPA-loaded PLGA NPs (0.5 mg/mL NPs).

Results: PVA-NPs encapsulating tPA had a mean size of ~350 nm, with a surface charge of -35 mV, while DMAB-NPs showed a mean size of 450 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 plateau at ~25% over the first 24h of release, which is similar to those obtained in a study by Chung *et al.*[7], with DMAB-NPs showing a more gradual release of tPA, compared to PVA-NPs. DMAB-NPs exhibited stronger binding to fibrin clots, compared to PVA-NPs (Fig. 1), which was attributed to electrostatic interactions between the cationic NPs and fibrin, which is negatively-charged[7] at pH 7.4.

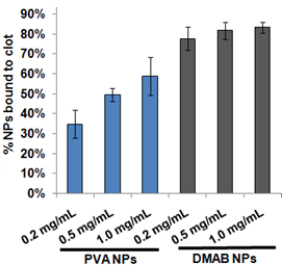

Figure 1. Clot binding data for PVA & DMAB NPs.

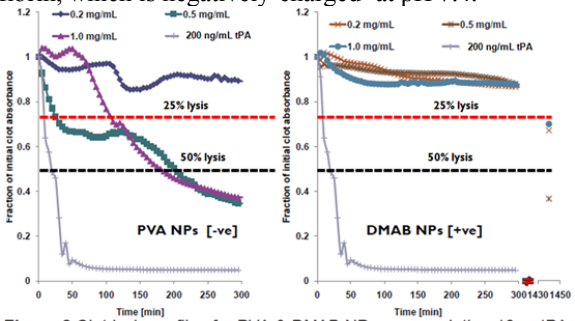

Figure 2. Clot lysis profiles for PVA & DMAB NPs encapsulating 10µg tPA.

NPs loaded with 10µg tPA showed more gradual tPA release and controlled fibrinolysis, compared to those loaded with 20 & 50 µg tPA. DMAB-NPs showed slower fibrinolysis compared to PVA-NPs (Fig.2), due to its slower tPA release. We hypothesize that their enhanced binding to the fibrin clot would enable their localization at the top-edge of the clot, leading to slower fibrinolysis.

In vitro EaRASMC proliferation was not significantly affected following fibrinolysis using 10 µg tPA-loaded PLGA NPs. However, fibrinolysis caused a significant decrease in elastic matrix deposition by EaRASMCs to levels observed prior to clot formation & lysis (Fig.3A) compared to the untreated clot control. This may be due to plasmin or MMPs. Although western blots showed no significant difference in MMP-2 synthesis (Fig.3B), gel zymography (Fig. 3C) revealed significant attenuation of MMP-2 & -9 activities following NP-based fibrinolysis. This suggests that fibrinolytic products may attenuate MMP activity, and the post-fibrinolytic decrease in elastic matrix deposition could be attributed to plasmin.

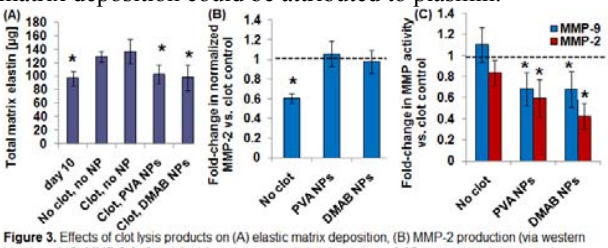

Figure 3. Effects of clot lysis products on (A) elastic matrix deposition, (B) MMP-2 production (via western blots), and (C) MMP-2 & -9 activity (via gel zymography). * denotes $p < 0.05$ vs. untreated clot control.

Ongoing studies seek to examine the role of plasmin via fibrinogen zymography[9], clot architecture via microscopy, and whole blood clot lysis experiments. Planned *in vivo* studies will provide insights into the feasibility of this NP-based modality in regulating the AAA environment for subsequent delivery of AAA therapy.

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**. Sivaraman *et al.* Acta Biomater 2013;9: 6511-25. **7.** Chung *et al.* Biomaterials 2008;29: 228-237. **8.** Undas *et al.* Blood. 2009;114: 4272-8. **9.** Ahmann *et al.* Tissue Eng.A 2010;16: 3261–70.