

Supporting Information for Publication

Cationic PAMAM Dendrimers Aggressively Initiate Blood Clot Formation (nn-2012-03472r)

Supplemental Figure 1. Cationic proteins form aggregates with anionic dendrimers.

Supplemental Figure 2. Vascular distribution of G7-NH₂-FITC in ZFE injected with various G7-NH₂-FITC doses.

Supplemental Figure 3. Heparin and warfarin have little effect on thrombotic phenotype observed in G7-NH₂-injected zebrafish.

Supplemental Video 1. Human thrombin initiates clotting in zebrafish embryos.

Supplemental Video 2. Prophylactic sodium citrate treatment blocks thrombin-induced clotting in zebrafish embryos.

Supplemental Figure Legends

Figure 1. Cationic proteins form aggregates with anionic dendrimers. Protamine, a small cationic protein (5-10kDa), does not form spontaneous aggregates on its own (lane 1) or in the presence of G7-NH₂ PAMAM dendrimer (lane 2). However, addition of G6.5-COOH dendrimer, an anionic dendrimer, results in the formation of protamine aggregates (lane 3, see arrow). Note: products of 1:340 dendrimer-protamine molar ratio (estimated ratio necessary for a protamine monolayer) are shown here.

Figure 2. Vascular distribution of G7-NH₂-FITC in ZFE injected with various G7-NH₂-FITC doses. ZFE (4 dpf, wildtype AB) were injected with saline, 1, 5, or 10 ng (A-D) of G7-NH₂-FITC. As in Figure 5, dose-dependent occlusion is evident in the tails of the zebrafish based on G7-NH₂-FITC staining in a similarly punctate fashion. Auto-fluorescence in uninjected ZFE as well as injected ZFE is due to the embryonic yolk sac.

Figure 3. Heparin and warfarin have little effect on thrombotic phenotype observed in G7-NH₂-injected zebrafish. One nL of heparin (15 mg/ml) was pre-injected into ZFE 2 hours prior to G7-NH₂ injection. In additional studies, heparin was injected 30 minutes before and immediately prior to G7-NH₂ dendrimer treatment (3 ng). Independent of the time of injection, treatment with heparin did not reduce occlusions (A). Zebrafish were presoaked in warfarin (500 μ M) up to 12 hours before G7-NH₂ dendrimer injection (3 ng). As depicted, warfarin did not block blood vessel occlusion as depicted (B). Prophylactic treatments of these magnitudes were observed to produce spontaneous bleeding at dendrimer injection sites.

Supplemental Video 1. Human thrombin initiates clotting in zebrafish embryos. ZFE (3 dpf, gata-1) were anesthetized, mounted, and imaged with a Leica M165 FC fluorescent microscope as described in **Methods**. ZFE were injected with 300 microunits of thrombin. One representative embryo is shown with the injection occurring at the beginning of the video.

Supplemental Video 2. Prophylactic sodium citrate treatment blocks thrombin-induced clotting in zebrafish embryos. ZFE were pre-soaked in 0.38% sodium citrate (in E3 embryo water) for 2 hours. ZFE (3 dpf, gata-1) were anesthetized, mounted, and imaged with a Leica M165 FC fluorescent microscope as described in **Methods**. ZFE were injected with 300

microunits of thrombin. Normal blood flow was observed following thrombin injection in approximately half of all embryos tested. One representative embryo is shown with the injection occurring at the beginning of the video.