Supplemental Material

New functional tools for anti-thrombogenic activity assessment of live surface glycocalyx

Authors

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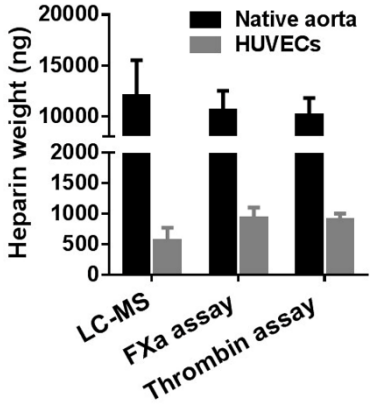
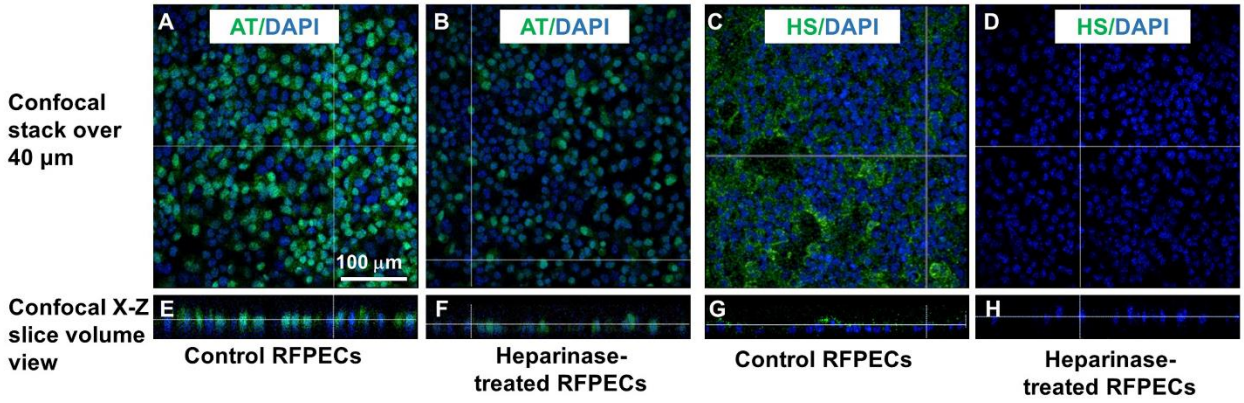


Supplemental Figure I. Comparison between different assays for detecting heparin weight on live surfaces. Total weight of heparin equivalent per cm^2 live surfaces (native aorta and HUVEC monolayer) was measured by LC-MS, FXa assay and thrombin assay. The results are mean of five independent assays using five different rats and HUVEC cultures (n=5).



Supplemental Figure II: Confocal images of the glycocalyx layer on rat fat pad endothelial cell (RFPEC) monolayer. (A, B, E, F) HS is visualized by fluorescein-labeled AT (green). (C, D, G, H) HS is visualized by anti-HS antibody (green). Also shown is DAPI costaining (blue). The X-Z plane side view showing the depth of the glycocalyx in RFPEC monolayer (E, G) and heparinase-treated RFPECs (G, H).

Supplemental Table I. Calculation of inactivated FXa using heparin standard

Heparin weight (ng)	Absorbance at 16 min	Residual FXa (Unit)	Inactivated FXa (Unit)
0	**0.3**	2	**0**
10	**0.21**	1.4	**0.6**
100	**0.18**	1.2	**0.8**
1000	**0.14**	0.93	**1.07**
10 000	**0.04**	0.25	**1.75**

Absorbance from FXa assay using heparin standard shown in Figure 1A

Supplemental Table II. Calculation of inactivated thrombin using heparin standard

Heparin weight (ng)	Absorbance at 11 min	Residual thrombin (Unit)	Inactivated thrombin (Unit)
0	**0.15**	0.5	**0**
10	**0.13**	0.42	**0.08**
100	**0.09**	0.29	**0.21**
1000	**0.07**	0.23	**0.27**
10 000	**0.05**	0.19	**0.31**

Absorbance from thrombin assay using heparin standard shown in Figure 2A