

## **Supplementary Material**

### **Dissolving porcine and human microthrombi by short exposure to microdoses of alteplase in an in vitro model of microvascular obstruction**

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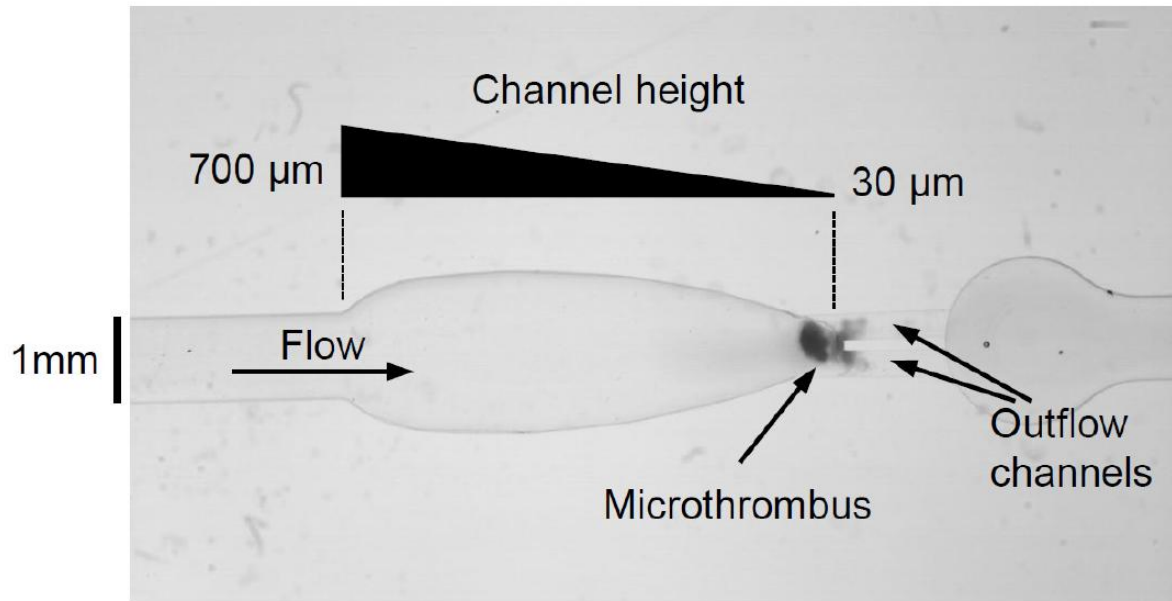
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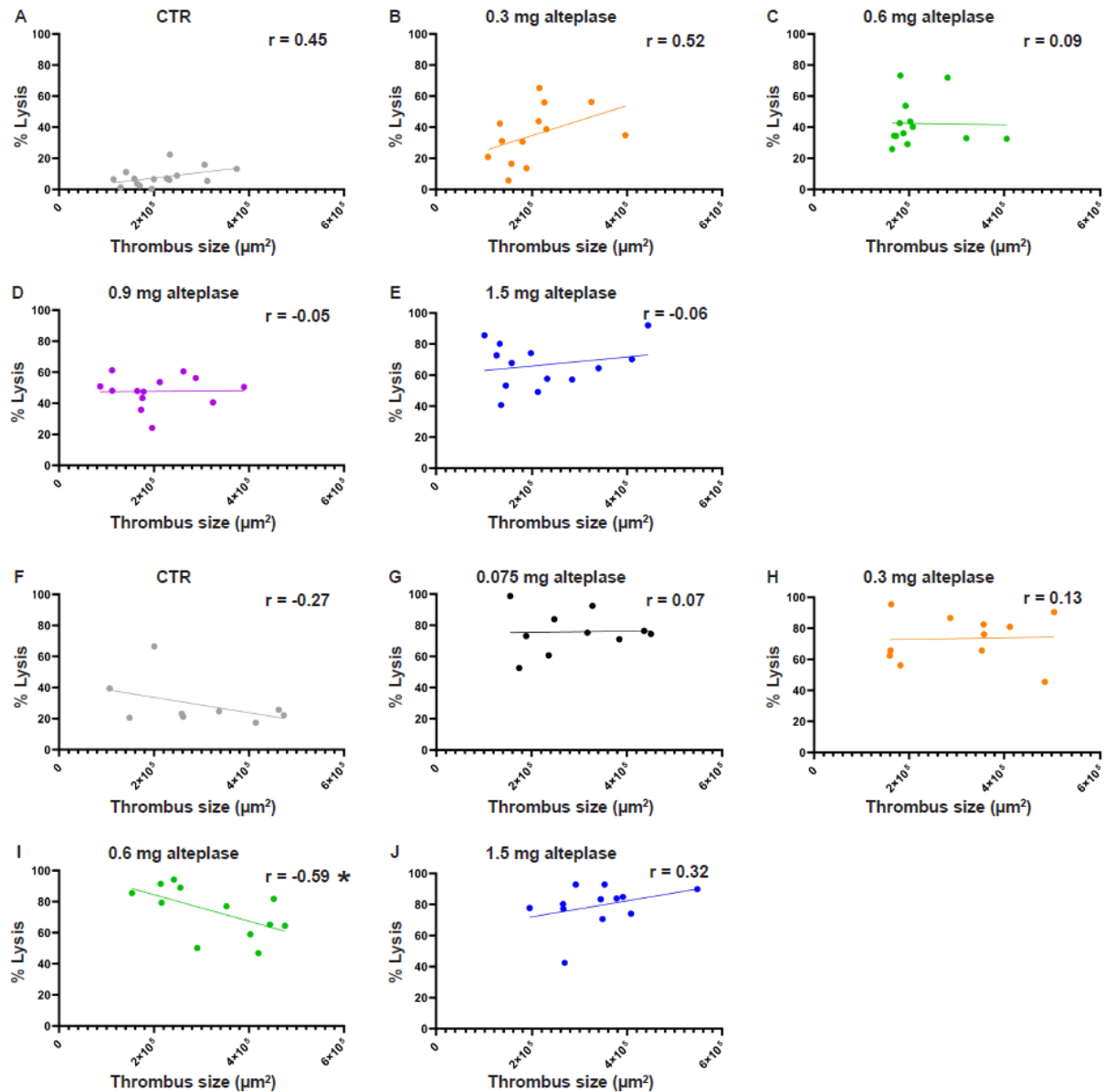
A	Alteplase microdose	Slope of clot dissolution		
		CoFI	CoFI + IV alteplase	p-value
	Control	0.0023 ± 0.00027	0.0035 ± 0.00037	<0.0001
	0.3 mg	0.013 ± 0.00085	0.02 ± 0.00062	<0.0001
	0.6 mg	0.014 ± 0.00056	0.019 ± 0.00048	0.85
	0.9 mg	0.021 ± 0.00070	0.028 ± 0.00051	0.0162
	1.5 mg	0.034 ± 0.00075	0.046 ± 0.00083	<0.0001

B	Alteplase microdose	Slope of clot dissolution		
		CoFI	CoFI + IV alteplase	p-value
	Control	0.013 ± 0.00048	0.052 ± 0.000955	<0.0001
	0.075 mg	0.03 ± 0.00095	0.04 ± 0.00085	<0.0001
	0.3 mg	0.032 ± 0.00102	0.04 ± 0.00095	0.0001
	0.6 mg	0.03 ± 0.00074	0.044 ± 0.00111	0.0203
	1.5 mg	0.035 ± 0.0013	0.041 ± 0.0012	<0.0001

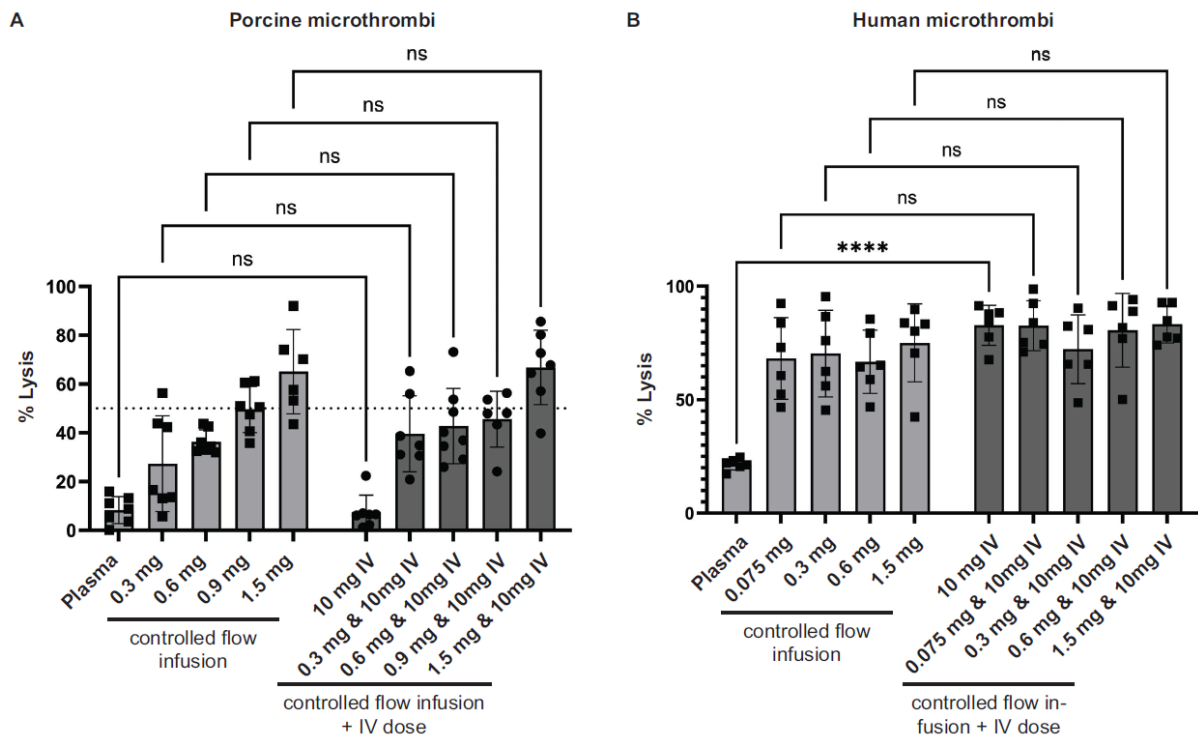
**Supplementary Table S1: Slope of thrombolysis for porcine and human microthrombi over time.** Slope of thrombolysis was calculated from lysis over time curves for **(A)** porcine microthrombi and **(B)** human microthrombi incubated with four different microdoses of alteplase or without alteplase (Control). Slopes for controlled flow infusion (CoFI) are compared to the combined approach (CoFI + IV dose). Values for slope steepness and standard deviation are shown. Data are from six to eight independent experiments and five to six different blood donors. Unpaired Student's t-test was used for statistical analysis and  $p < 0.05$  was considered significant.



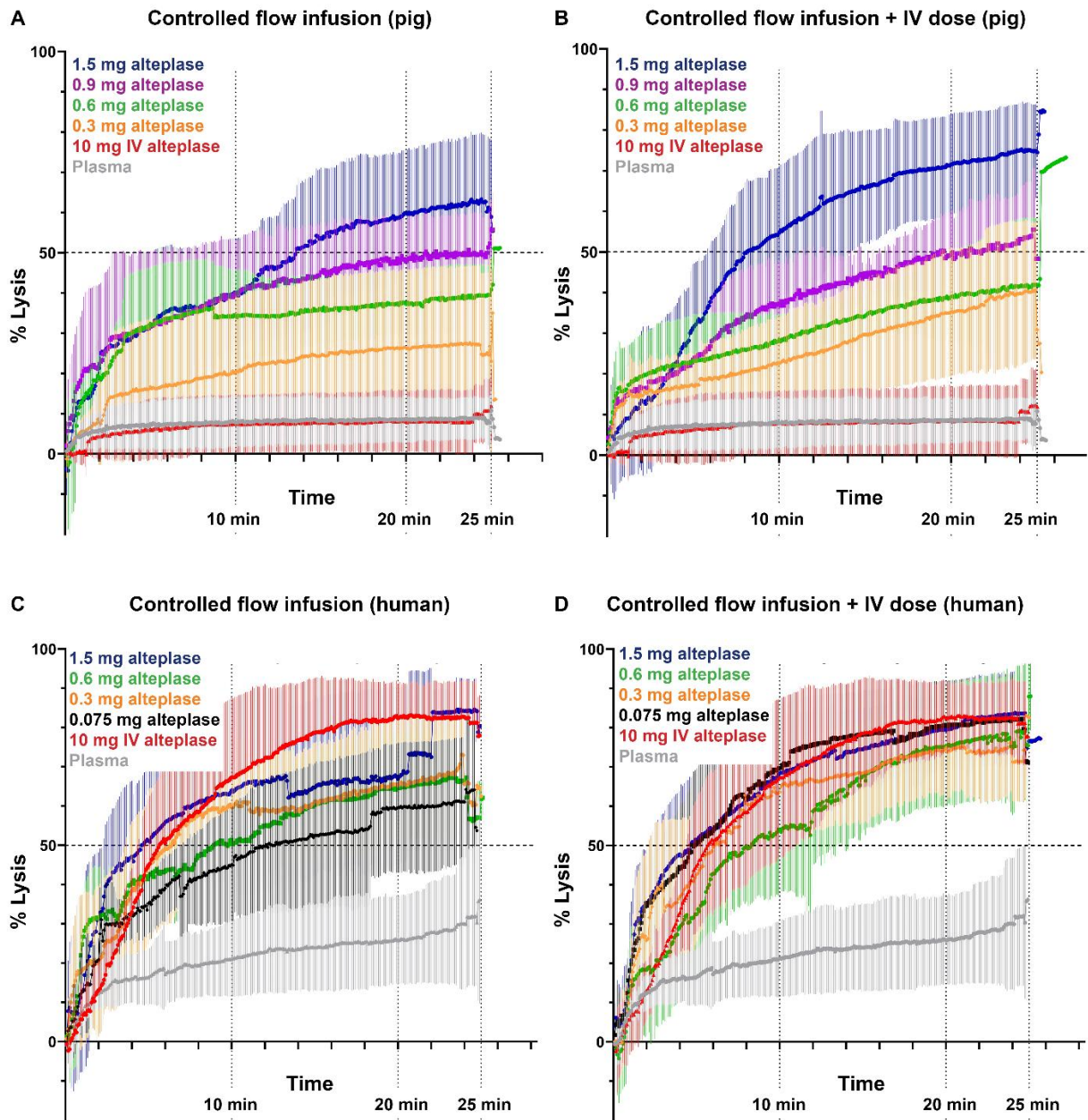
**Supplementary Figure S1: Microfluidic chip design to model microvascular obstruction in vitro.** Image of the microfluidic chip with a chamber of tapered height (channel height 700  $\mu\text{m}$  – 30  $\mu\text{m}$ ) in which a microthrombus is trapped. Two outflow channels (300  $\mu\text{m}$  wide and 30  $\mu\text{m}$  high) assure continuous perfusion without complete channel occlusion after MT injection. Upon perfusion, flow occurs from left to right and is maintained at a constant flow rate of 37  $\mu\text{l}/\text{min}$  using a syringe pump.



**Supplementary Figure S2: Relationship between initial thrombus size and microthrombus lysis.** (A-E) porcine MT and (F-J) human MT are plotted according to the MT size in  $\mu\text{m}^2$ , and the corresponding MT lysis achieved at the end of lysis experiments. Linear regression analysis and nonparametric correlation test (Spearman  $r$ ) was used to determine whether initial MT size had an influence on the amount of lysis for all tested alteplase microdoses as well as controls (not treated with alteplase).  $p < 0.05$  was considered significant.



**Supplementary Figure S3: MT lysis comparing CoFI to a combined approach.** MT lysis after 25 minutes perfusion with citrate plasma (light grey bars) or citrate plasma containing 2  $\mu$ g/ml alteplase (dark grey bars) for porcine (**A**) or human (**B**) MT. Two-way ANOVA with multiple comparisons was used for statistical analysis. Data are from six to eight independent experiments and five to six different blood donors. \*\*\*\*  $p < 0.001$



**Supplementary Figure S4: Standard deviation of thrombolysis of MT over time comparing controlled flow infusion to a combined approach (CoFI + IV dose). (B, C) Thrombolysis over time for (A, B) porcine or (C, D) human MT treated with different alteplase microdoses and perfused with (A, C) plasma or (B, D) plasma containing 2  $\mu\text{g}/\text{ml}$  alteplase. Each curve represents the mean lysis over time of at least 6 independent experiments with the corresponding standard deviation.**