Supporting Information

Nebulisation of RNA-Loaded Micelle-Embedded Polyplexes as a Potential Treatment of Idiopathic Pulmonary Fibrosis

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Table S1 from Zimmermann et al, doi: 10.1016/j.jconrel.2022.09.021. Sequences of siRNAs used in the study. Nt = nucleotides; GFP = green fluorescence protein; NC = negative control; GAPDH = housekeeping gene GAPDH; A = Adenine; C = Cytosine; G = Guanine; U = Uracil; T = Thymine; p = phosphate residue; lower case bold letters = 2´-deoxyribonucleotides; capital letters = ribonucleotides; underlined capital letters = 2´-O-methylribonucleotides.

Name	Sense strand (5'-3')	Antisense strand (3'-5')	Length (nt)	
			Sense	Antisense
siGFP	pACCCUGAAGUUCAUCUG CACCAC**cg**	ACUGGGACUUCAAGUAGAC GUGGUGGC	**25**	**27**
siNC	pCGUUAAUCGCGUAUAAU ACGCGU**at**	CAGCAAUUAGCGCAUAUUA UGCGCAUAp	**25**	**27**
siGAPDH	pGGUCGGAGUCAACGGAU UUGGUC**gt**	UUCCAGCCUCAGUUGCCUA AACCAGCA	**25**	**27**

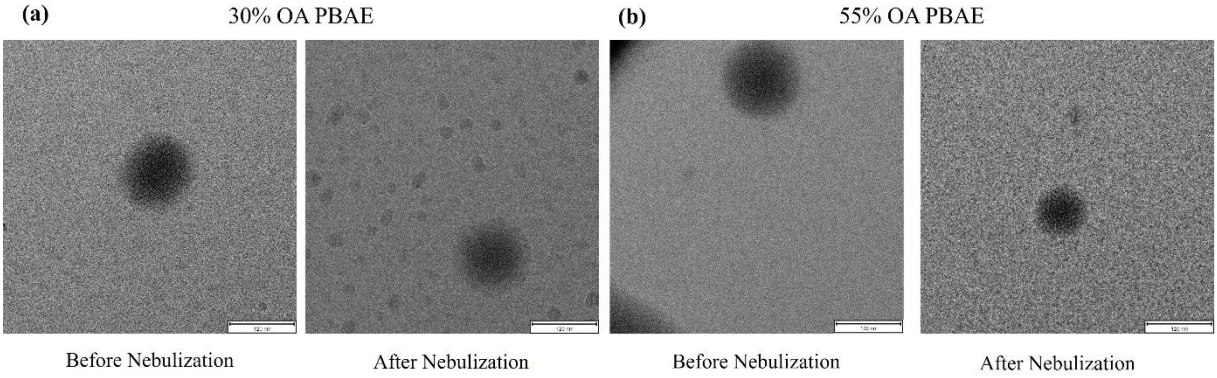

Figure S1: Cryo-TEM pictures of 30% OA (a) and 55% OA (b) PBAEs mPolyplexes before (left) and after nebulisation (right)

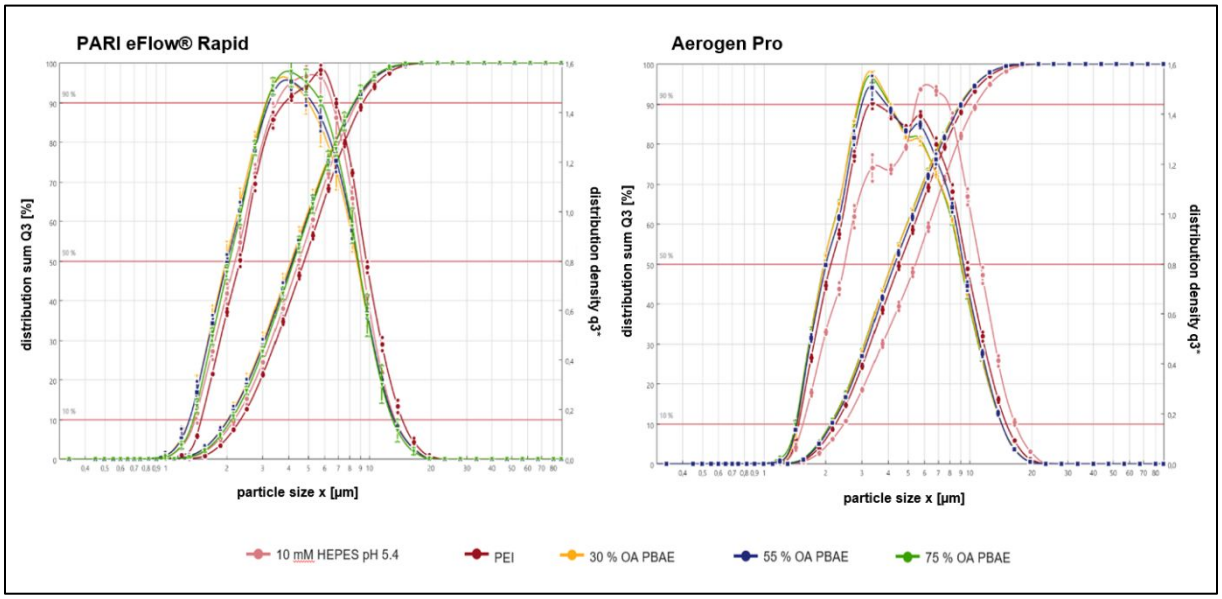

Figure S2: Laser diffraction results of nebulised formulations and control solutions.

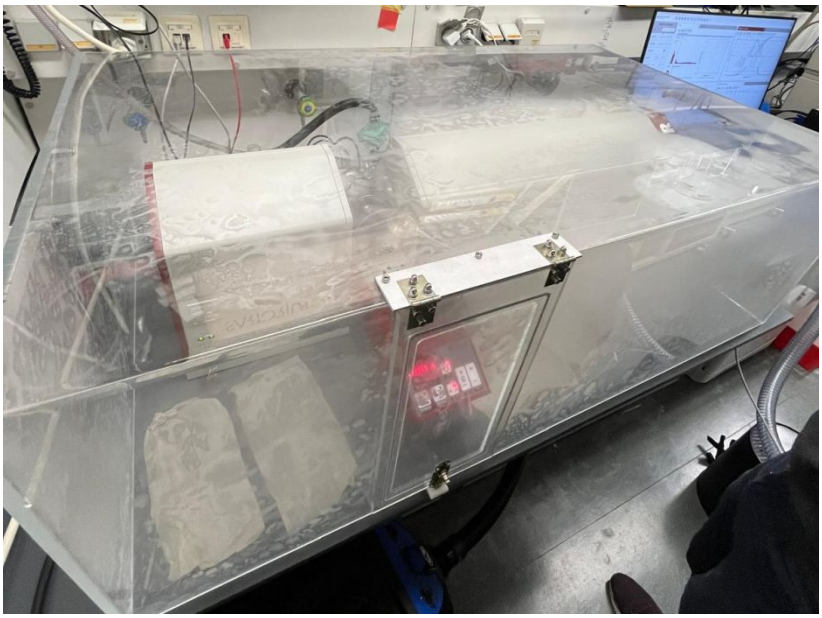

Figure S3: Experimental set-up with equipped humidity box.

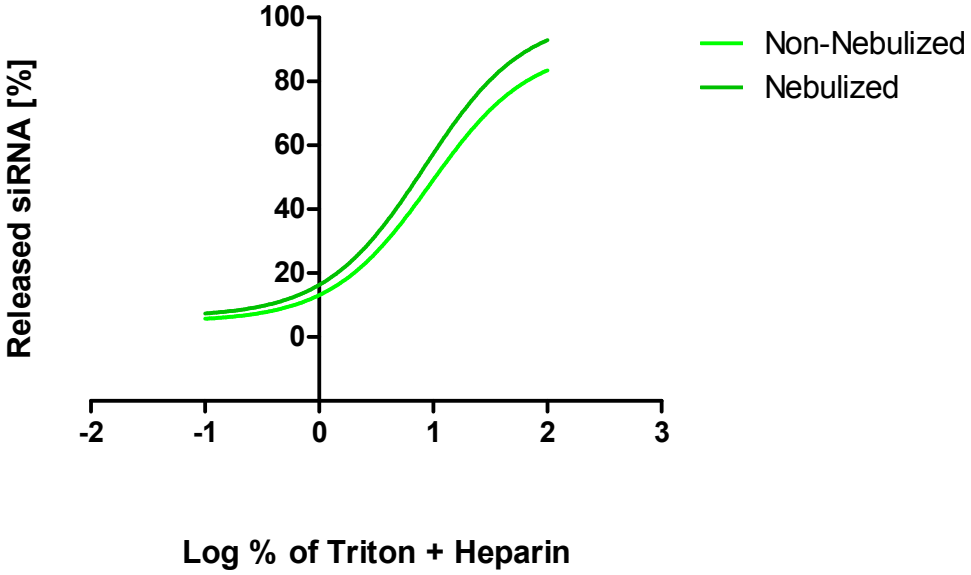

Figure S4: Intraparticular stability of 30% OA NP´s determined via siRNA release as a function of of Triton-X and heparin concentrations.

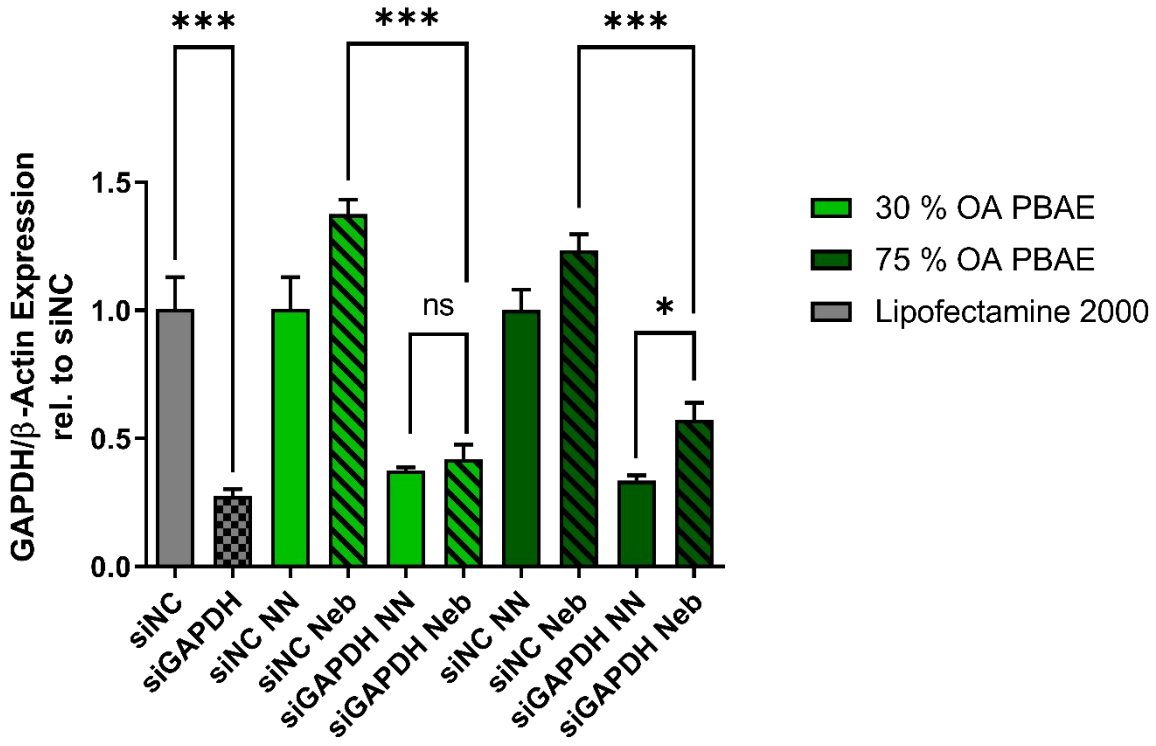

Figure S5: GAPDH knockdown in peritumour PCLS after transfection with mPolyplexes made of PBAEs with different OA content before and after nebulisation.

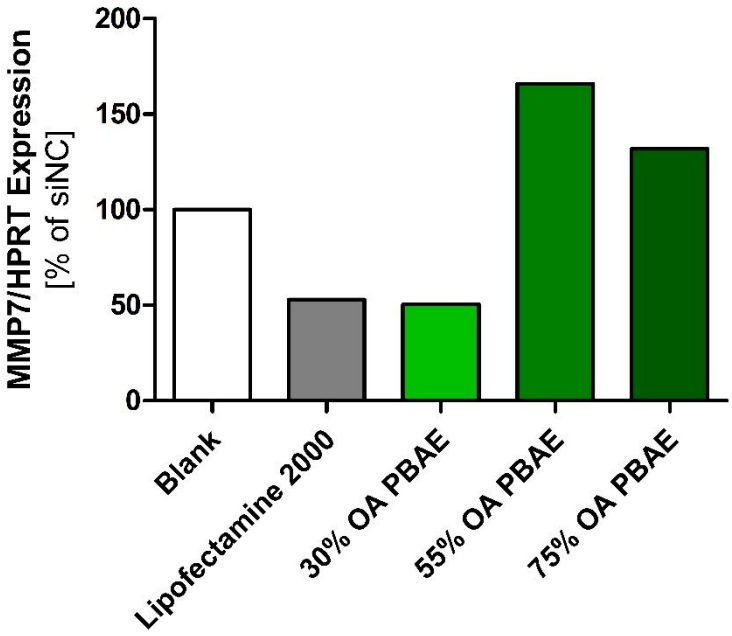

Figure S6: MMP7 Knock-Down Screening in fibrotic PCLS with mPolyplexes consisting of PBAEs with different OA content.

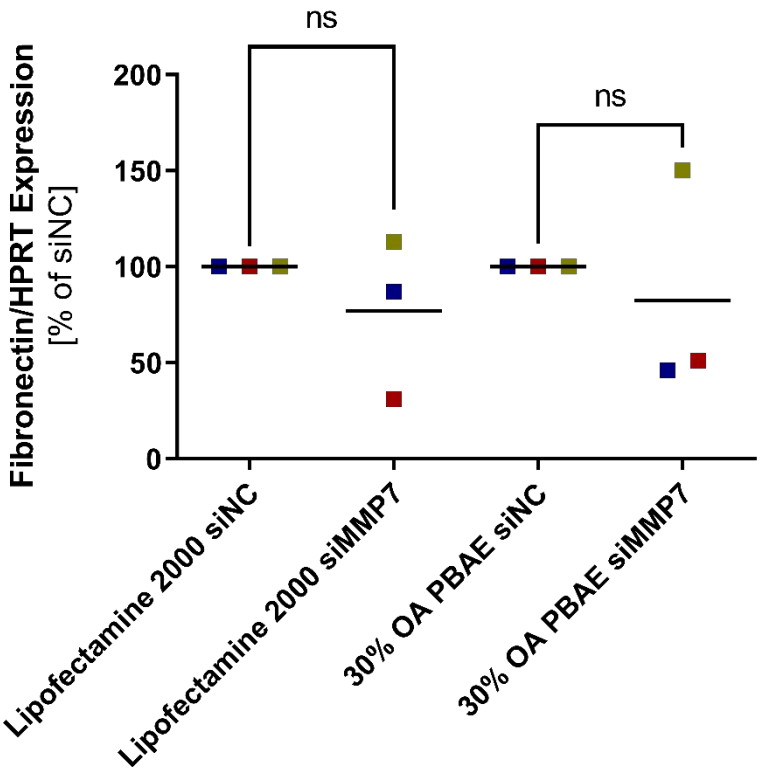

Figure S7: Fibronectin knockdown in fibrotic PCLS transfected with Lipofectamine 2000 and nebulised 30% OA PBAEs mPolyplexes.

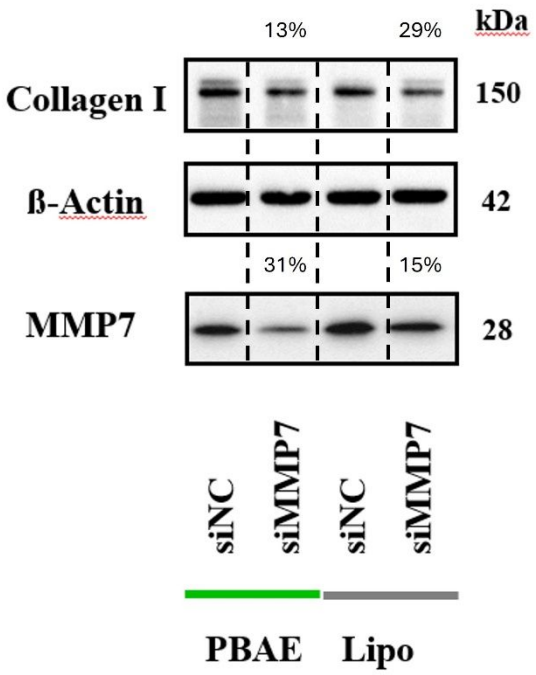

Figure S8: Collagen I and MMP-7 knockdown after transfection with Lipofectamine 2000 and nebulised 30% OA PBAEs. Percentages are shown against negative control sequence and all bands are corrected for β-actin bands intensity as housekeeping gene.

CMC determination of the polymers:

PBAE stocks were diluted in 10 mM HEPES pH 5.4 to concentrations between 0.1 and 200 µg/mL. Fluorescence emission spectra (Figure S9 and S10.) were recorded for each concentration using a plate reader (TECAN Spark, TECAN, Männedorf, Switzerland)) between 300 and 450 nm excitation and 500 nm emission wavelength.

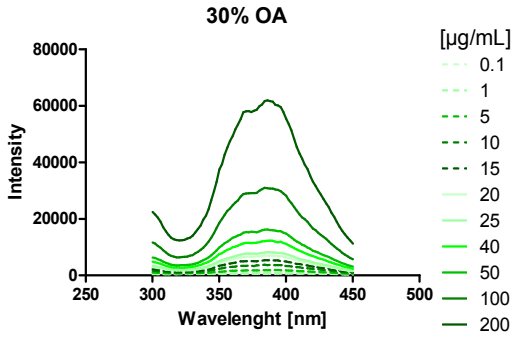

Figure S9. Exemplary fluorescence emission spectra recorded at 500 nm for different concentrations of 30% OA PBAE solutions in 10 mM HEPES pH 5.4

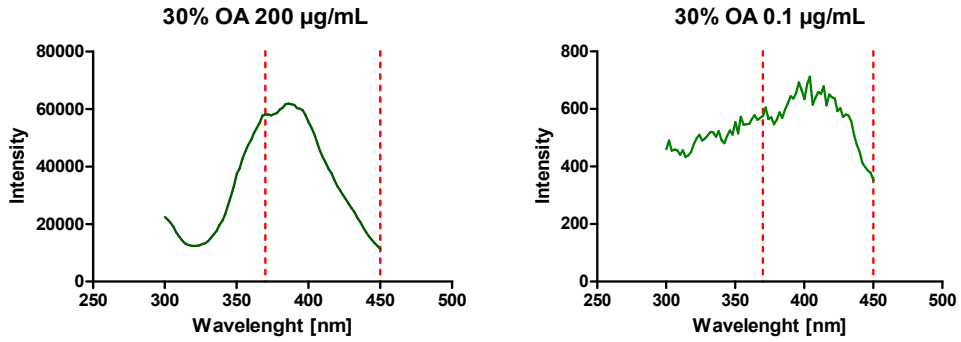

Figure S10. Difference in fluorescence emission spectra above (left) and below (right) the CMC.

To calculate the CMC, the ratio between fluorescence intensities between 450 nm and 370 nm was plotted against the polymer concentrations (Figure S11).

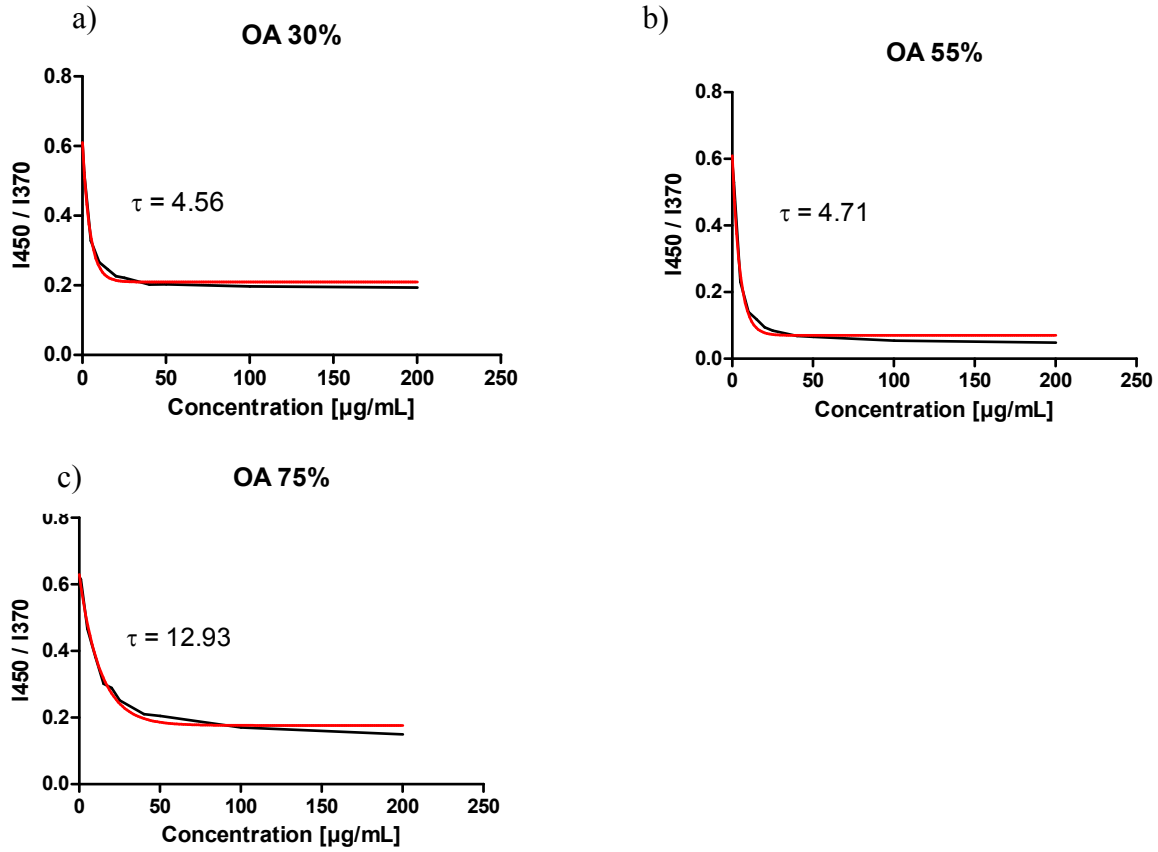

Figure S11. Intensity ratios from fluorescence spectra plotted against PBAE concentrations for a) 30%, b) 55%, and c) 75% OA. Depicted are the exponential decay curve fit (red) and measurement data (black).

The time constant τ was extrapolated from the resulting curve fits and depicts the determined CMC. Noteworthy, the CMC values vary only slightly from each other in the investigated OA range. Since all nanoparticle formulations used in the study were prepared at concentrations exceeding the respective CMC by orders of magnitude, the differences between them were neglected in this study.