

# Biological In Vitro Evaluation of PIL Graft Conjugates: Cytotoxicity Characteristics

Katarzyna Niesyto<sup>1</sup>, Wiktoria Łyżniak<sup>1</sup>, Magdalena Skonieczna<sup>2,3,\*</sup> and Dorota Neugebauer<sup>1,\*</sup>

<sup>1</sup> Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian University of Technology, 44-100 Gliwice, Poland; katarzyna.niesyto@polsl.pl

<sup>2</sup> Department of Systems Biology and Engineering, Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland

<sup>3</sup> Biotechnology Centre, Silesian University of Technology, Krzywoustego 8, 44-100 Gliwice, Poland

\* Correspondence: Magdalena.Skonieczna@polsl.pl (M.S.); Dorota.Neugebauer@polsl.pl (D.N.)

## Content:

**Figure S1.** Microscopic images by Live Cell Analyzer of untreated control cells vs treated (a) A549 and (b) BEAS-2B cells by polymer I and IV with  $\text{Cl}^-$ ,  $\text{PAS}^-$  and  $\text{CLV}^-$  counterions;

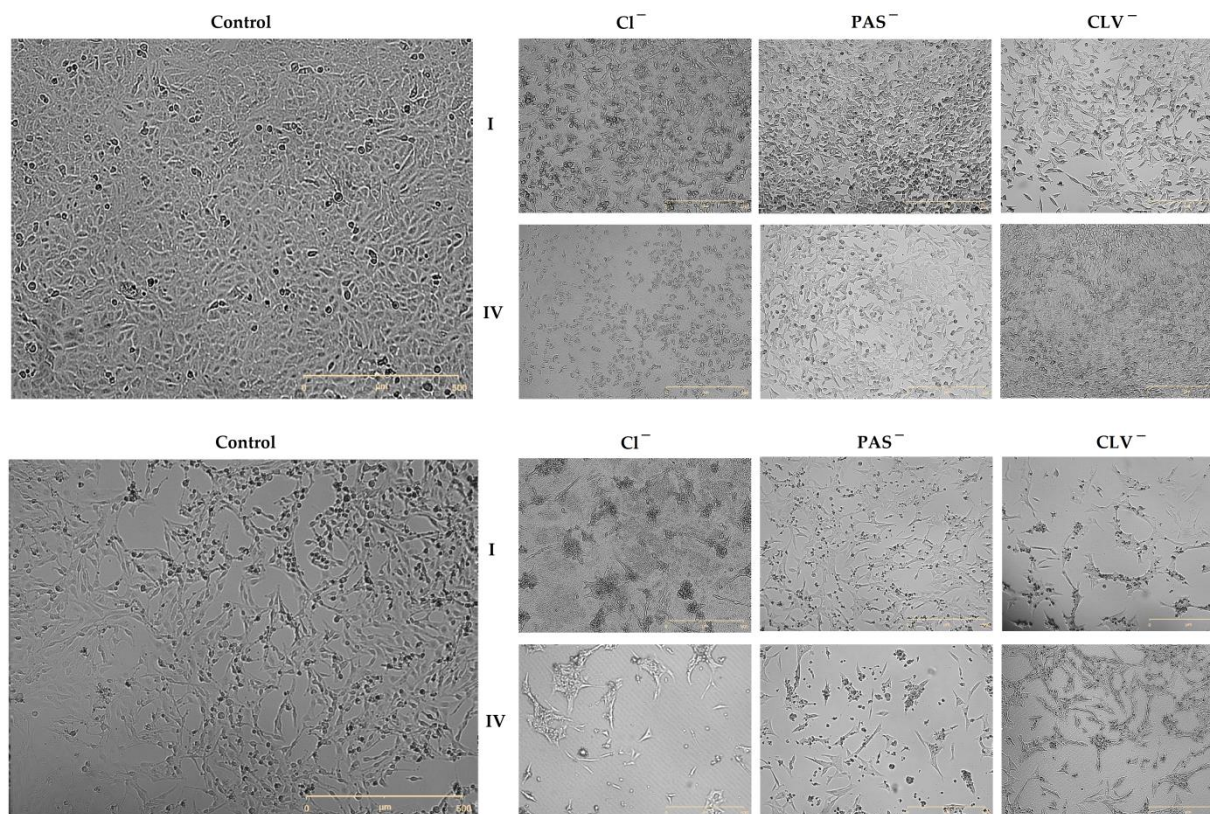
**Table S1.** Results of Annexin V apoptosis assay in A549 cells for control probe, PIL carriers varying with content of TMAMA units (25% and 50%) and grafting degree (26% and 46%), and their conjugates with PAS and CLV;

**Table S2.** Results of Annexin V apoptosis assay in BEAS-2B cells for control probe, PIL carriers varying with content of TMAMA units (25% and 50%) and grafting degree (26% and 46%), and their conjugates with PAS and CLV;

**Figure S2.** Plots of I and IV cell populations determined by flow cytometric analysis in (a) A549 and (b) BEAS-2B cell line;

**Table S3.** Results of cell cycle analysis in A549 cells for control probe, PIL carriers varying with content of TMAMA units (25% and 50%) and grafting degree (26% and 46%), and their conjugates with PAS and CLV;

**Table S4.** Results of cell cycle analysis in BEAS-2B cells for control probe, PIL carriers varying with content of TMAMA units (25% and 50%) and grafting degree (26% and 46%), and their conjugates with PAS and CLV.



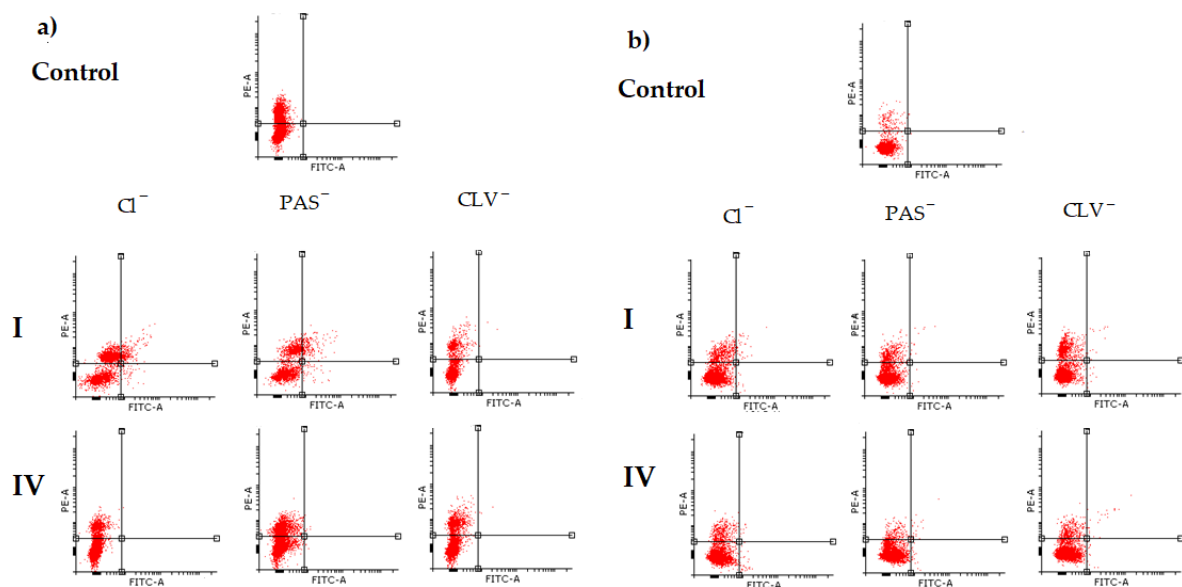
**Figure S1.** Microscopic images by Live Cell Analyzer of untreated control cells vs treated (a) A549 and (b) BEAS-2B cells by polymer I and IV with  $\text{Cl}^-$ ,  $\text{PAS}^-$  and  $\text{CLV}^-$  counterions

**Table S1.** Results of Annexin V apoptosis assay in A549 cells for control probe, PIL carriers varying with content of TMAMA units (25% and 50%) and grafting degree (26% and 46%), and their conjugates with PAS and CLV

	Carrier				Conjugates with:							
					PAS				CLV			
	A- /PI- (%)	A+ /PI- (%)	A- /PI+ (%)	A+ /PI+ (%)	A- /PI- (%)	A+ /PI- (%)	A- /PI+ (%)	A+ /PI+ (%)	A- /PI- (%)	A+ /PI- (%)	A- /PI+ (%)	A+ /PI+ (%)
Control	78.7	0	21.3	0.04	78.7	0	21.3	0.04	78.7	0	21.3	0.04
I	42.2	0.8	48.8	8.3	65.3	1.8	21.9	11.0	92.3	0	7.6	0.03
II	32.9	1.7	48.1	17.3	69.5	0	30.5	0.03	82.9	0	17.1	0.03
III	32.4	1.9	53.0	12.7	82.0	0	17.9	0.01	72.6	0	27.4	0.01
IV	75.2	0.3	19.8	4.7	76.0	1	21.2	1.8	86.9	0	13.0	0.05

**Table S2.** Results of Annexin V apoptosis assay in BEAS-2B cells for control probe, PIL carriers varying with TMAMA content (25% and 50%) and grafting degree (26% and 46%), and their conjugates with PAS and CLV

	Carrier				Conjugates with:							
					PAS				CLV			
	A- /PI- (%)	A+ /PI- (%)	A- /PI+ (%)	A+ /PI+ (%)	A- /PI- (%)	A+ /PI- (%)	A- /PI+ (%)	A+ /PI+ (%)	A- /PI- (%)	A+ /PI- (%)	A- /PI+ (%)	A+ /PI+ (%)
Control	97.5	0.1	2.2	0.2	97.5	0.1	2.2	0.2	97.5	0.1	2.2	2.2
I	90.3	0.5	8.3	0.9	93.7	0.1	6.0	0.1	89.8	0.8	8.7	0.7
II	85.5	0.7	13.2	0.6	90.5	0.7	9.6	0.3	89.0	1.0	9.3	0.8
III	89.1	1.1	9.3	0.5	87.5	0.5	11.7	0.3	88.2	1.6	9.5	0.7
IV	93.4	1.0	5.2	0.4	92.8	0.7	6.2	0.3	90.1	0.8	8.7	0.5



**Figure S2.** Plots of I and IV cell populations determined by flow cytometric analysis in (a) A549 and (b) BEAS-2B cell line.

**Table S3.** Results of cell cycle analysis in A549 cells for control probe, PIL carriers varying with content of TMAMA units (25% and 50%) and grafting degree (26% and 46%), and their conjugates with PAS and CLV.

	Carrier				Conjugates with:							
					PAS				CLV			
	Sub-G1 (%)	G0/G1 (%)	S (%)	G2/M (%)	Sub-G1 (%)	G0/G1 (%)	S (%)	G2/M (%)	Sub-G1 (%)	G0/G1 (%)	S (%)	G2/M (%)
Control	42.51	17.62	36.07	3.80	42.51	17.62	36.07	3.80	42.51	17.62	36.07	3.80
I	0.75	69.56	16.78	12.61	65.3	70.36	19.40	7.35	37.06	7.77	52.06	3.13
II	0.25	75.26	16.12	8.24	69.5	44.25	39.04	14.32	4.54	26.76	63.63	5.07
III	0.41	66.76	23.86	8.96	82.0	43.35	44.89	6.34	17.23	18.35	60.29	4.13
IV	2.82	68.40	18.95	9.82	76.0	68.40	18.95	9.82	2.95	43.04	48.75	5.24

**Table S4.** Results of cell cycle analysis in BEAS-2B cells for control probe, PIL carriers varying with content of TMAMA units (25% and 50%) and grafting degree (26% and 46%), and their conjugates with PAS and CLV.

	Carrier				Conjugates with:							
					PAS				CLV			
	Sub-G1 (%)	G0/G1 (%)	S (%)	G2/M (%)	Sub-G1 (%)	G0/G1 (%)	S (%)	G2/M (%)	Sub-G1 (%)	G0/G1 (%)	S (%)	G2/M (%)
Control	34.86	17.51	44.74	2.88	34.86	17.51	44.74	2.88	34.86	17.51	44.74	2.88
I	1.95	6.80	75.98	15.27	3.59	12.51	73.14	10.77	0.66	6.58	80.12	12.65
II	0.96	4.91	69.20	24.92	0.94	7.68	56.81	34.77	0.96	6.67	63.30	29.07
III	1.18	12.51	63.47	22.93	1.44	8.76	77.29	12.51	1.22	7.87	65.98	24.93
IV	2.12	13.52	75.78	8.57	1.92	15.48	75.13	7.47	3.92	16.06	71.56	8.46