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Anionic peptide coating as a modification of PEI-based polyplexes for successful gene delivery

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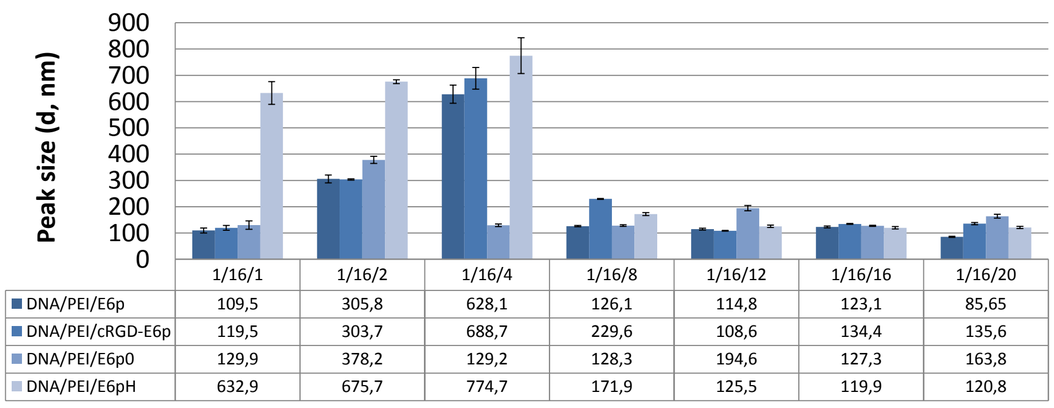
INTRODUCTION

Polyethylenimine (PEI) is one of the most studied molecules for non-viral delivery of nucleic acids into cells. The widespread use of PEI is limited because of instability of its polyplexes in the presence of serum components, as well as toxic effects. Modification of PEI polyplexes with charge-shielding coating will overcome the limitations of transfection in the presence of serum components. We developed anionic peptide coating modified with αvβ3 integrin ligand for targeted delivery. The purpose of this work was to study DNA/PEI polyplexes with anionic peptide coating as a means of delivering DNA into cells.

METHOD

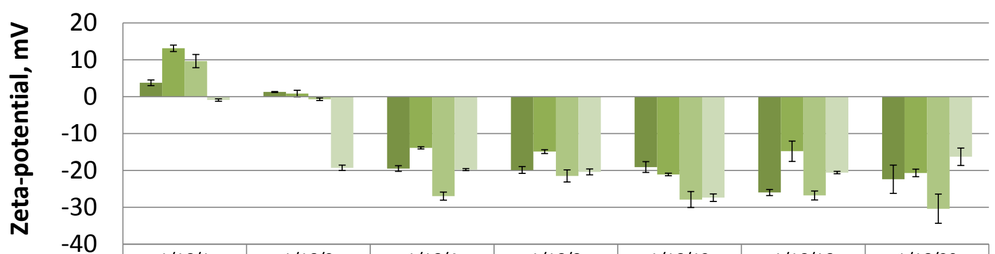
Glutamate-rich peptides modified with cycloRGD ligand were synthesized. Physicochemical properties of anionic coated DNA-PEI-polyplexes were tested. Size and zeta-potential of the resulting polyplexes was assessed by DLS and microelectrophoresis, respectively. The toxicity of formed complexes to PANC-1 cells was assessed by measuring metabolic activity (AlamarBlue test). Polyplexes stability was evaluated in transfection experiments in the presence of serum on PANC-1 cells. Transfection efficiency was assessed by *lacZ* reporter gene expression biochemically and by flow cytometry analysis of *GFP*-expressing cells.

RESULTS & DISCUSSION



	1/16/1	1/16/2	1/16/4	1/16/8	1/16/12	1/16/16	1/16/20
DNA/PEI/E6p	109,5	305,8	628,1	126,1	114,8	123,1	85,65
DNA/PEI/cRGD-E6p	119,5	303,7	688,7	229,6	108,6	134,4	135,6
DNA/PEI/E6p0	129,9	378,2	129,2	128,3	194,6	127,3	163,8
DNA/PEI/E6pH	632,9	675,7	774,7	171,9	125,5	119,9	120,8

Fig.1. Sizes of formed PEI-polyplexes (diameter, nm)



	1/16/1	1/16/2	1/16/4	1/16/8	1/16/12	1/16/16	1/16/20
DNA/PEI/E6p	3,75	1,26	-19,5	-19,9	-19,1	-26	-22,4
DNA/PEI/cRGD-E6p	13,1	0,81	-13,9	-14,9	-21,1	-14,8	-20,7
DNA/PEI/E6p0	9,65	-0,698	-27	-21,5	-27,9	-26,8	-30,4
DNA/PEI/E6pH	-0,93	-19,3	-19,8	-20,4	-27,4	-20,6	-16,3

Fig.2. Zeta potentials of formed PEI-polyplexes (mV)

The sizes (fig.1) and zeta potentials (fig.2) of the formed polyplexes were measured. Interestingly, almost all polyplexes at the best charge ratio (1/16/4) were large in size (> 500 nm in diameter). Charge ratio of 1/16/4 is also the ratio at which the charge of polyplexes changes from positive to negative (except for polyplexes DNA/PEI/E6p0 (size) and DNA/PEI/E6pH (zeta potential)).

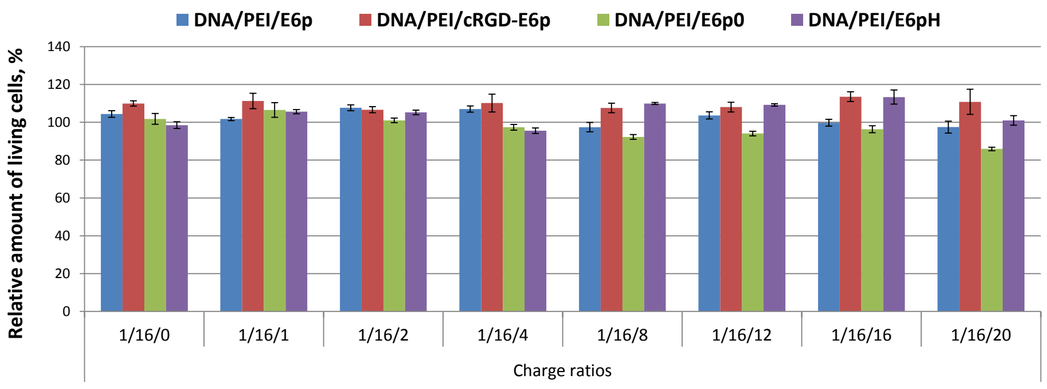
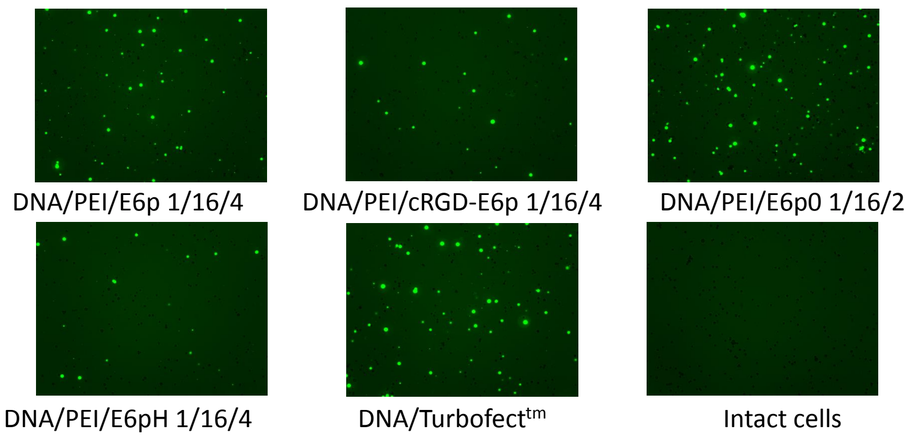


Fig.3. Amount of living cells (%) after transfection with PEI-polyplexes

Polyplexes with all studied anionic peptides at this charge ratio are nontoxic for PANC-1 cells (fig.3).



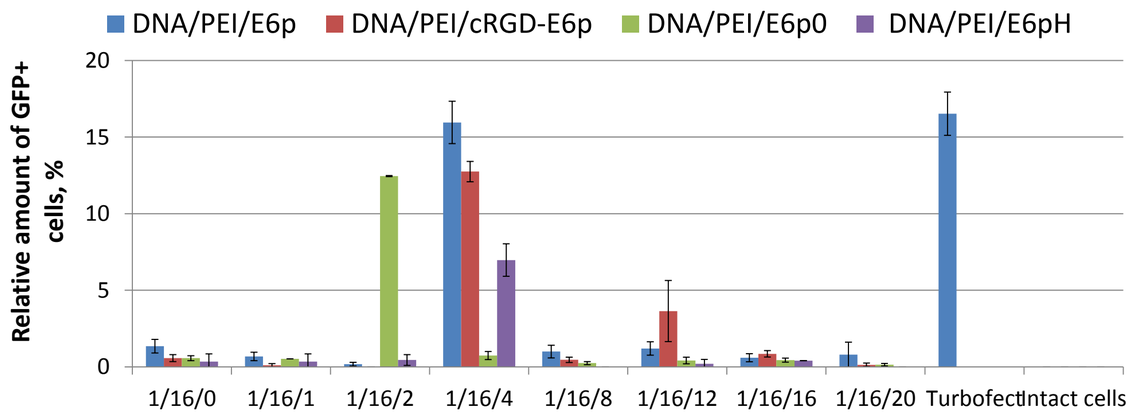


Fig.4. Transfection efficiency of PEI-polyplexes (cell counter)

Analysis of transfection efficiency by biochemical method and by flow cytometric analysis of cells showed comparable results, Figures 4 and 5 show the results of GFP detection

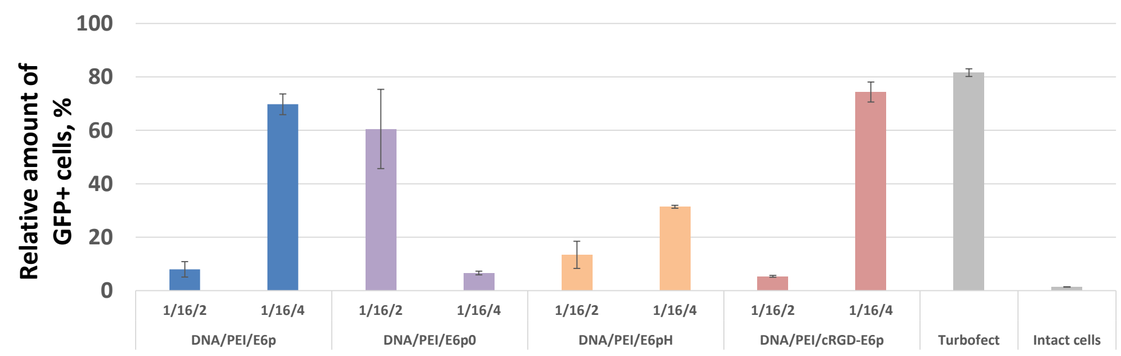


Fig.5. Transfection efficiency of PEI-polyplexes (flow cytometer)

CONCLUSION

Study shows that the utilization of anionic peptide coating with PEI-polyplexes promotes successful transfection in the presence of serum. Developed modification should be considered as a universal module of non-viral delivery systems.