The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

Study of physicochemical, toxic, and transfection properties of siRNA-polyplexes stabilized by anionic peptides

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Abstract:

Currently, gene therapy is considered as a potentially universal approach to the treatment of a wide range of diseases, such as cancer, cardiovascular diseases, diabetes, inherited diseases, and many others. It is known that “naked” nucleic acid is not able to effectively overcome the extra- and intracellular barriers to reach the target cell, since it is easily degraded. In this regard, it becomes necessary to create special delivery vehicles. We investigated the modular arginine-rich cysteine-flanked polycondensed carriers stabilized by anionic glutamic acid-rich peptides. Glutamic acid-rich peptides can give colloidal stability of the studied complexes and protect them from interaction with negatively charged components of blood serum.

Physicochemical, toxic, and transfection properties of the investigated complexes with siRNA were studied in cancer cell line MDA-MB-231-GFP^{+}. Condensation analysis showed that the complexes with optimal charge ratios are not destroyed by the addition of anionic peptides. Also these polyplexes were stable in the serum-containing culture medium during cell transfection and non-toxic for the cells. Moreover, we measured the average size and zeta-potential of the siRNA-nucleopeptide complexes. Based on this study, it was concluded that the complexes are capable of stably bind siRNA and have a high transfection efficiency in an optimal charge ratio, which provides new opportunities for the use of these carriers for siRNA delivery in vivo.

Keywords: anionic peptides; gene therapy; non-viral vectors; nucleopeptide complexes; siRNA.

Introduction

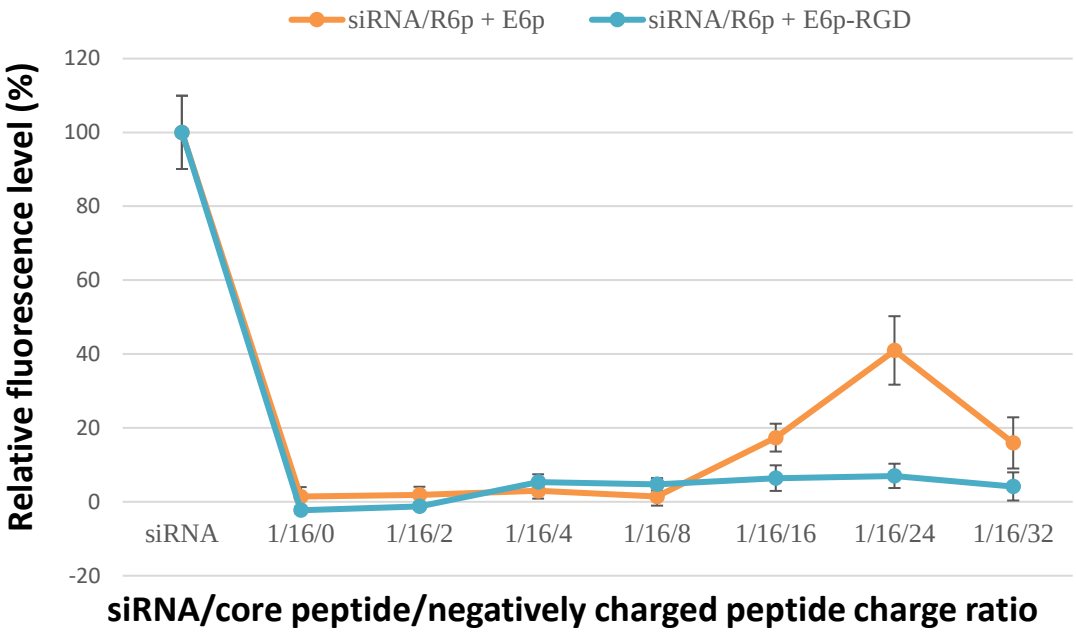
Application of non-viral peptide carriers as a delivery system seems to be one of the most perspective approaches for gene therapy. Such NA-based therapeutics have been researched well and have a number of advantages such as low immunogenicity and toxicity, biodegradability, and also their structure can be easily modified with ligand moieties.

Polycationic carriers are capable to interact with negatively charged nucleic acids electrostatically to form stable particles, that significantly increases the delivery efficiency *in vitro*. However, the positively charged cationic polymers interact with negatively charged serum proteins *in vivo*, that leads to the destruction of the particles. It is known that the anionic coating based on polyglutamic acid increases the transfection efficacy significantly, as it reduces the possibility of interaction with serum proteins. Thus, it is of importance to define the complex optimal charge ratios to be stable and have high transfection efficacy in the presence of fetal bovine serum.

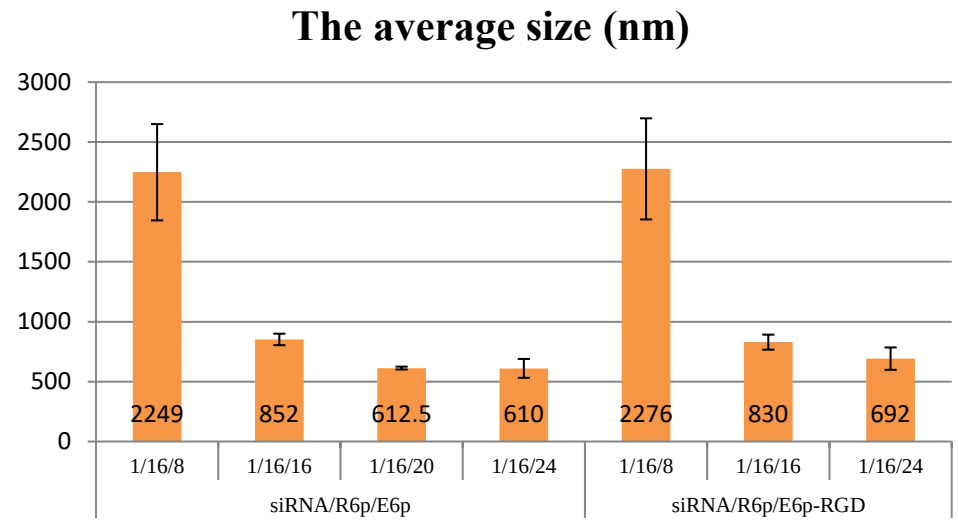
Results and discussion

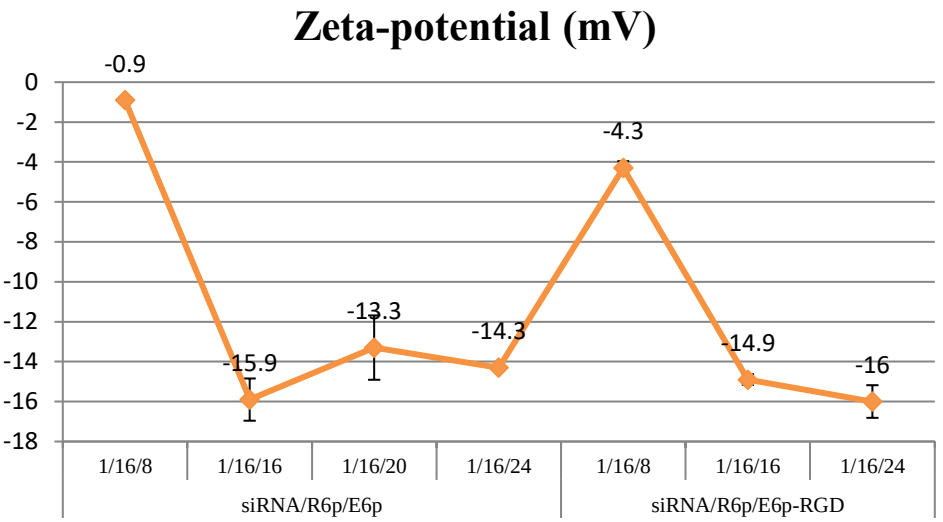
The graph shows the change in the fluorescence intensity of the SYBR Green with an increase in the siRNA/core peptide/negatively charged peptide charge ratios with standard deviation (S. D.).

The minimum charge ratio in which the studied complexes are stable is 1/16/8. It should be noted that the siRNA/R6p+E6p-RGD are more stable than the siRNA/R6p+E6p complexes.



Results and discussion

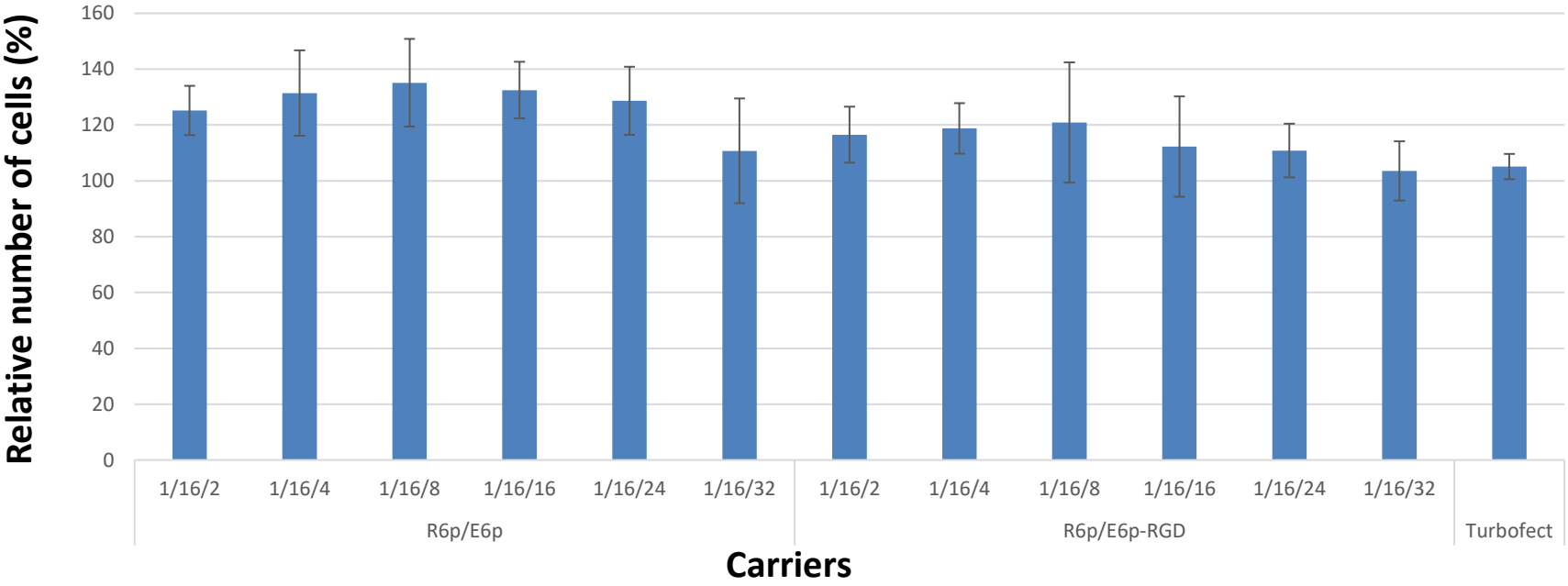




The graphs show the average size (left) and zeta-potential (right) of the investigated siRNA-nucleopeptide complexes with standard deviation (S. D.).

The average size and zeta-potentialof the complexes varies from 610 nm to 2276 nm and from -16 mV to -0,9 mV, respectively. It should be noted that an increase in the complexes charge ratios leads to a decrease in the average size and zeta potential of the complexes.

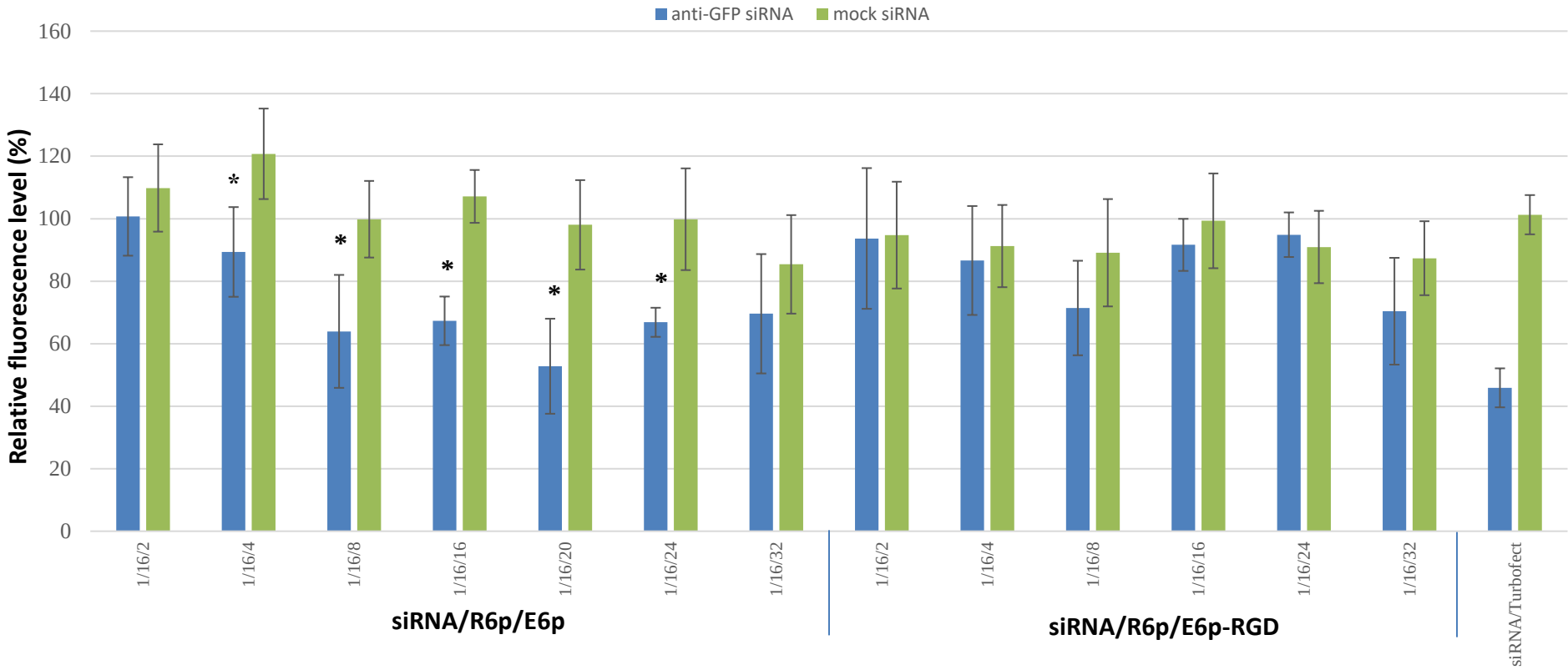
Results and discussion



The graph shows the relative number of cells (%) after transfection MDA-MB-231-GFP$^+$ cancer cell line with the studied siRNA-complexes with an increase charge ratios.

The toxicity of the complexes does not exceed the toxicity of the complexes with the commercial carrier Turbofect.

Results and discussion



The graph shows the change in the fluorescence intensity after transfection of the MDA-MB-231-GFP$^+$ cancer cell culture with anti-GFP siRNA/carrier/anionic peptide complexes ($^*p<0.05$).

The charge ratio of the complexes that demonstrate the highest transfection efficacy is 1/16/20. That can be compared with the transfection efficacy by complexes with the commercial carrier Turbofect.

Conclusions

The current study shows that the developed nucleopeptide complexes coating by anionic peptides allow stable interaction with siRNA, is not non-toxic, and results in the high transfection efficacy in the presence of fetal bovine serum with the optimal charge ratio. That provides new opportunities for using peptide-based siRNA delivery systems for gene therapy *in vivo*.

Grant RSF 21-15-00111