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# Study of electrostatically stabilized nucleopeptide complexes for targeted DNA delivery to muscle cells

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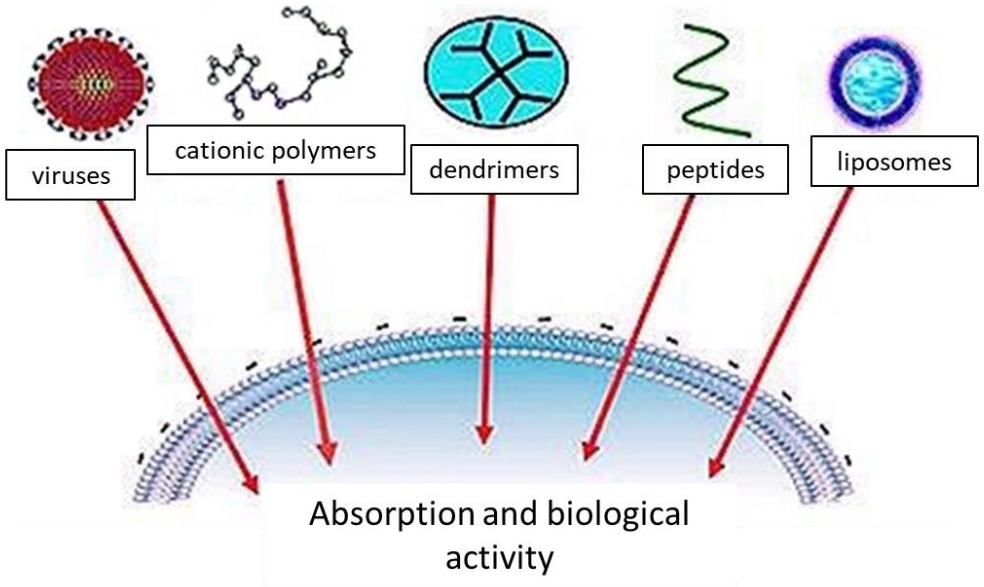
**Abstract**

Genetic constructs must be selectively delivered to target tissues and intracellular compartments in the necessary concentrations to achieve the maximum therapeutic effect in gene therapy. The search for ways to implement targeted non-viral delivery of nucleic acids into cells, including muscle cells, as one of the most difficult to transfect tissues in vivo, remains topical. We have developed coated cationic nucleopeptide complexes containing ASSLNIA ligand for targeted DNA delivery to muscle tissue.

**Keywords:** C2C12; gene therapy; mdx mice; peptide carriers.

# Delivery methods

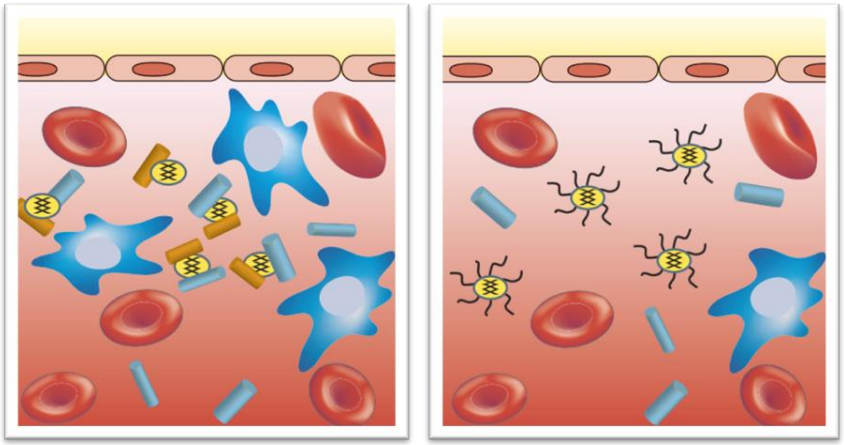
Virus-based delivery system:
- Immunogenicity
- The risk of accidental integration into the genome
- Difficulties in preparation
- High price



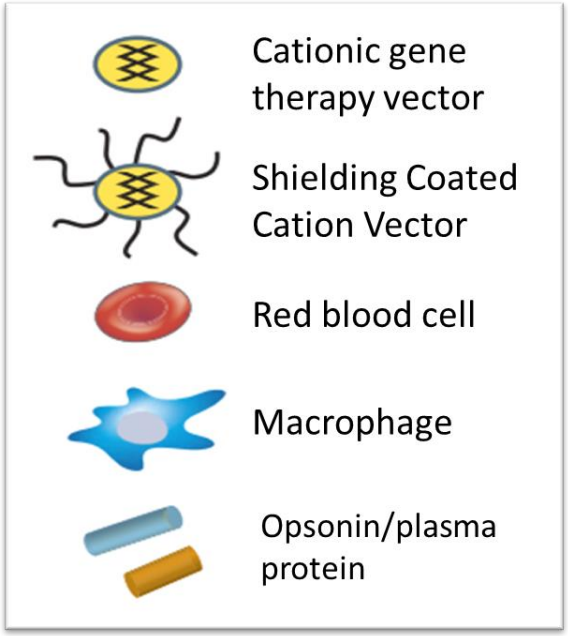

Peptide based delivery system:
+ Biodegradable
+ Homogeneous
+ Possibility of modification with various modules to overcome nucleic acid transport barriers

In experiments on mdx mice, which are a laboratory model of a hereditary neuromuscular disease - Duchenne muscular dystrophy, a significant therapeutic effect in muscle tissue has been achieved so far only using a virus-based delivery system (Li, Samulski, 2020), which, however, has a number of disadvantages. Non-viral carriers are an alternative to viral vectors. They are devoid of the above disadvantages.

# Overcoming extracellular barriers to nucleic acid transport



McErlean et al., 2016 (with changes)




Cationic peptide carriers are capable of electrostatically interacting with negatively charged DNA due to their positive charge. However, upon entering the blood serum, such complexes bind with opsonins and blood plasma proteins, which leads to the degradation of DNA-peptide complexes. The addition of a shielding coating makes the complexes inaccessible for interaction with plasma proteins, as a result of which their use becomes possible under these conditions.

**Our aim** is to study the properties of peptide carriers based on cationic and anionic peptides modified with a ligand for targeted DNA delivery to muscle cells in the presence of blood serum.

Objectives:

1. To study the toxic properties of DNA / carrier complexes.
2. To study the transfection properties of DNA / carrier complexes.
3. To analyze DNA condensation by a developed peptide carrier.
4. To study the packing properties of the carrier in the presence of polyanions (dextran sulfate).
5. To perform in vivo delivery of plasmid encoding GFP gene into *m. quadriceps* of mdx mice.
6. To evaluate the results of in vivo pDNA delivery using fluorescence microscopy.

Material : C2C12 myoblasts, mice mdx

Peptide carriers :

• R6pH, R6pHH - arginine-histidine-rich cysteine-containing polycondensed carriers.

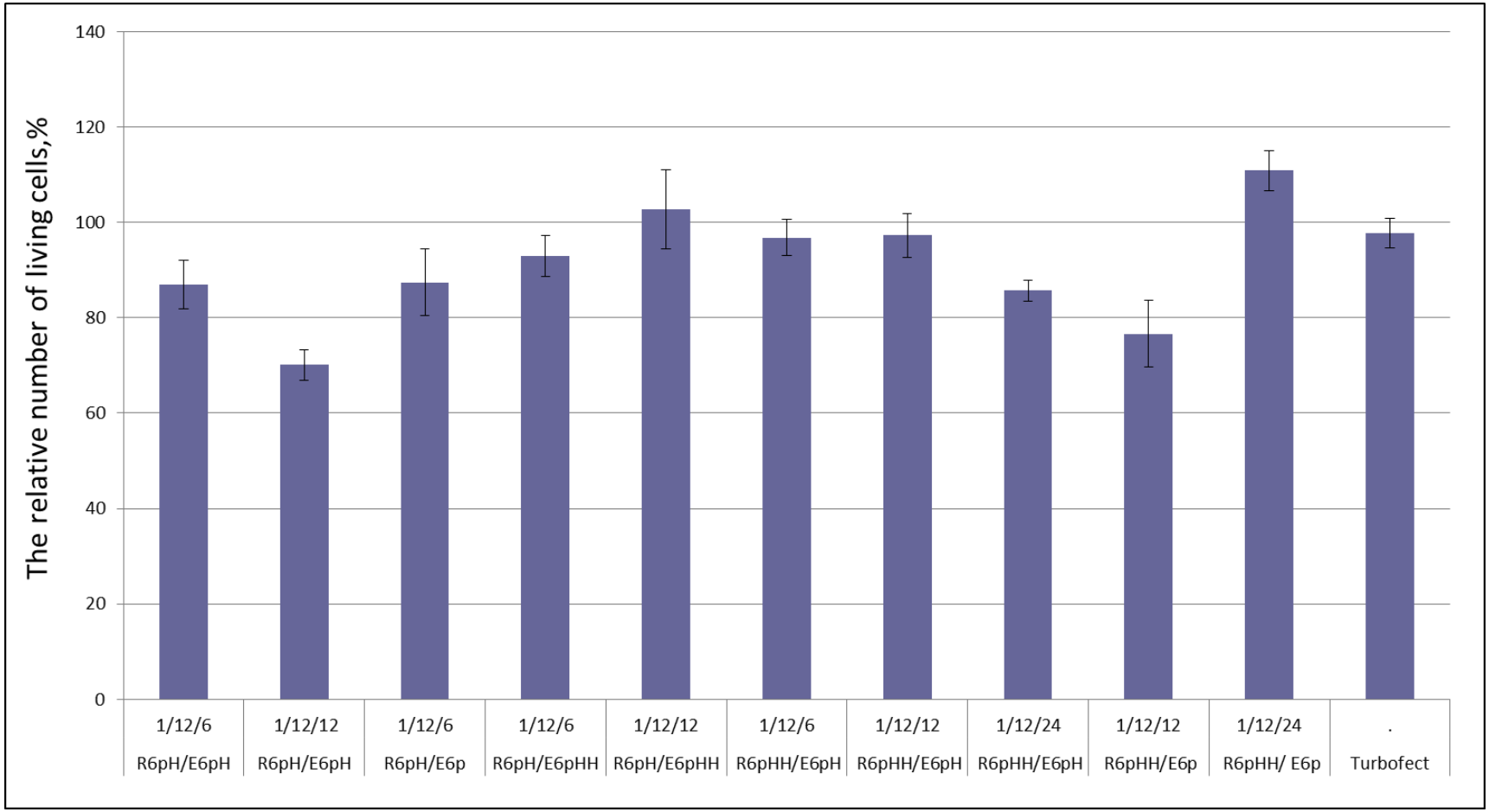
• E6p, E6pH, E6pHH - glutamine-histidine-rich cysteine-containing polycondensed carriers.

• ASSLNIA-ahx-ahx-E6pHH – E6pHH carrier modified with ligand for binding to muscle cells.

Methods :

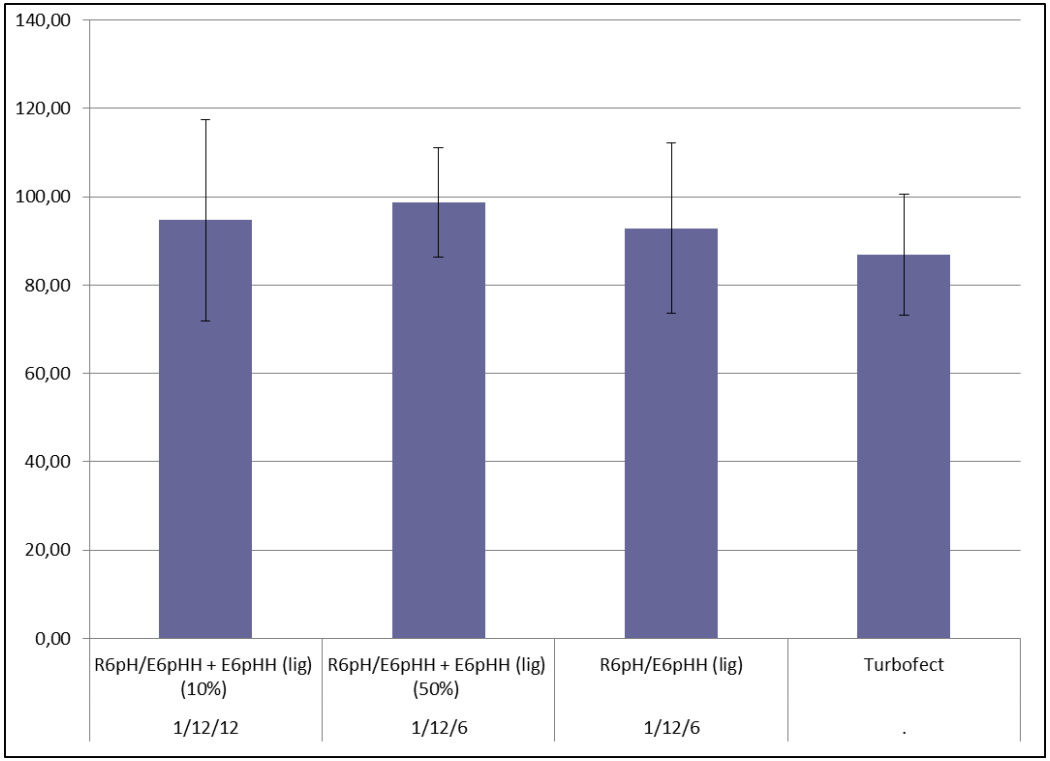
1. Study of toxic properties using the Alamar Blue test (resazurin test).
2. Study of the transfection properties of the complexes using the *lacZ* and *GFP* reporter genes.
3. Analysis of DNA condensation by peptide carriers using the EtBr displacement test.
4. Delivery of plasmid DNA with the *GFP* gene into *m. quadriceps* of mdx mice.
5. Cryosectioning and fluorescent microscopy analysis.

# Results of toxicity analysis using the Alamar Blue test



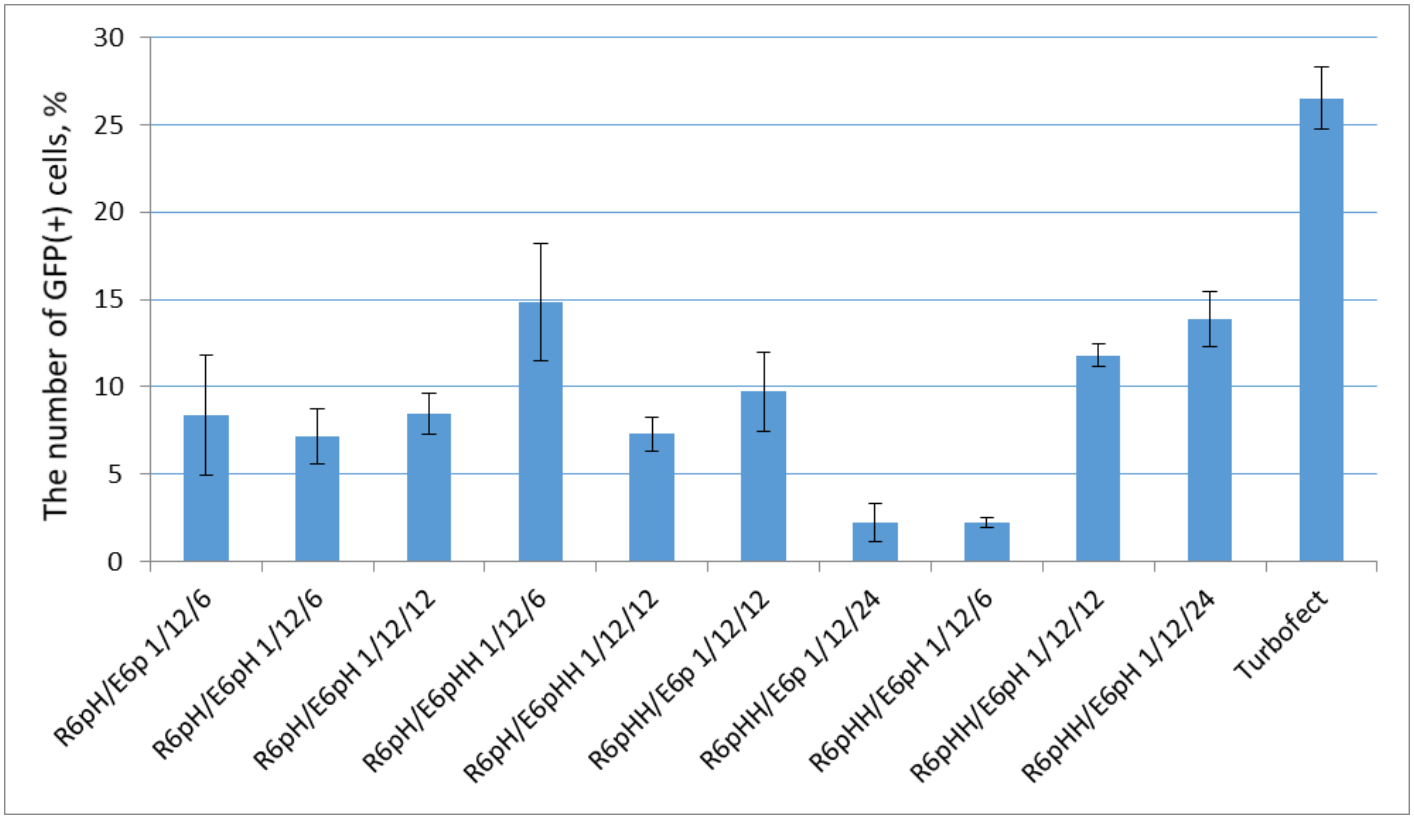

Percentage of viable C2C12 cells after transfection by DNA / carrier complexes at different DNA / carrier1 / carrier2 charge ratios. The standard error of the mean is indicated.

# Results of toxicity analysis using the Alamar Blue test



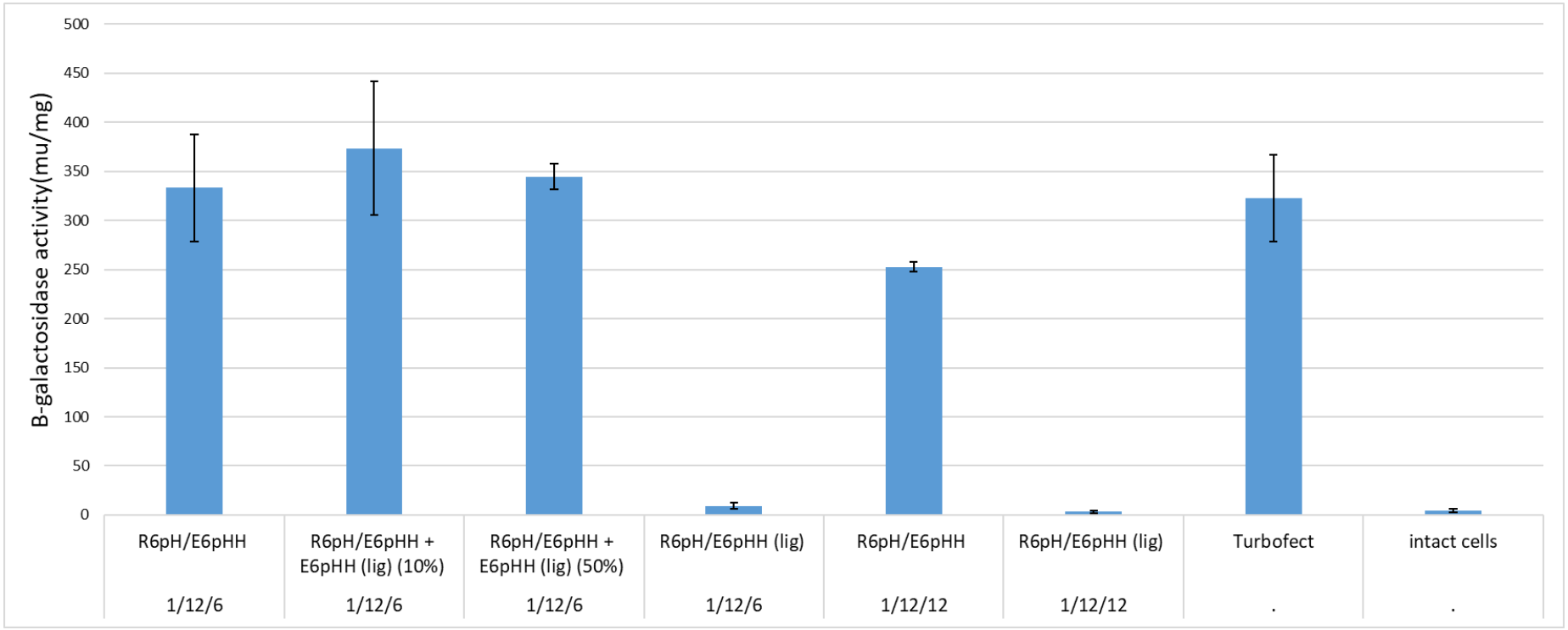

Percentage of viable C2C12 cells after exposure to DNA / carrier complexes at different DNA / carrier1 / carrier2 charge ratios with different ligand content (10 mol%, 50 mol%, 100 mol%). The standard error of the mean is indicated.

# Results of transfection of C2C12 myoblasts with plasmid DNA with the *GFP* gene delivered using peptide carriers



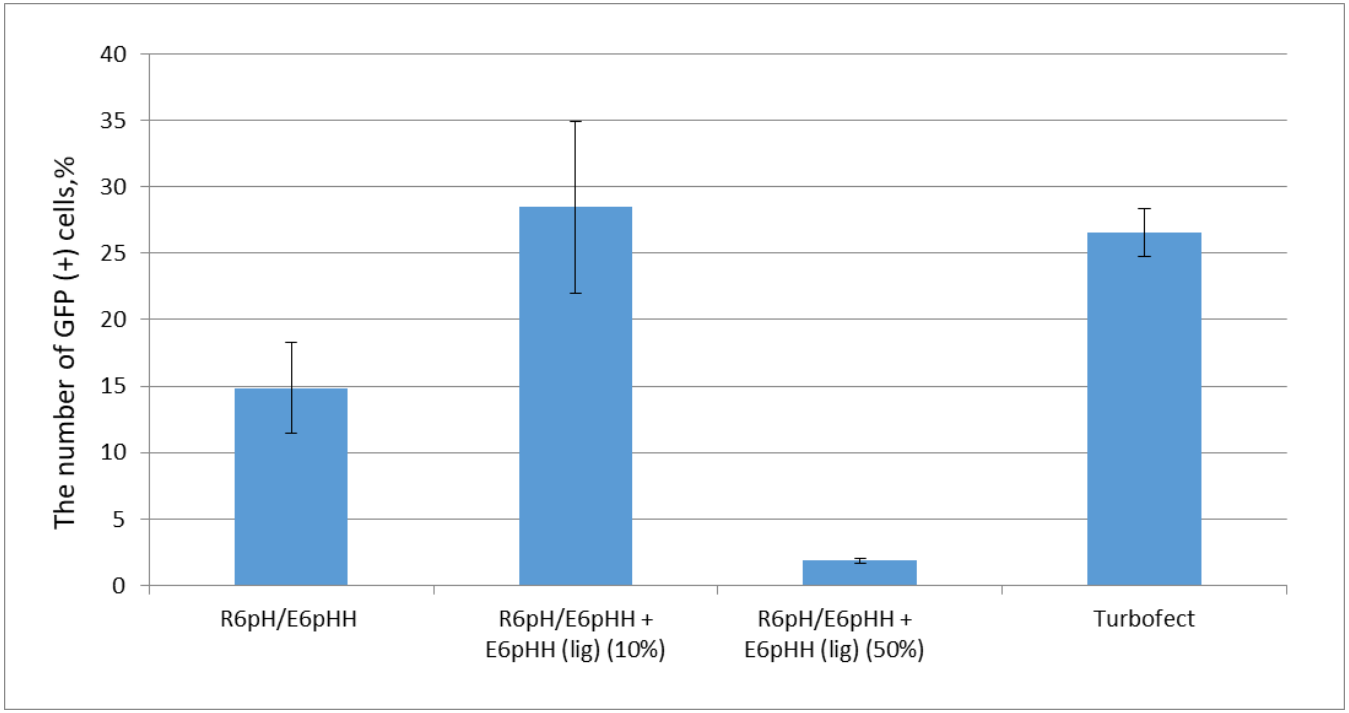

Relative number of GFP-positive cells after delivery of DNA using peptide carriers at various ratios of DNA / carrier1 / carrier2. Shown mean and standard error of the mean

# Results of transfection of myoblasts C2C12 with plasmid DNA with the *lacZ* gene delivered using peptide carriers



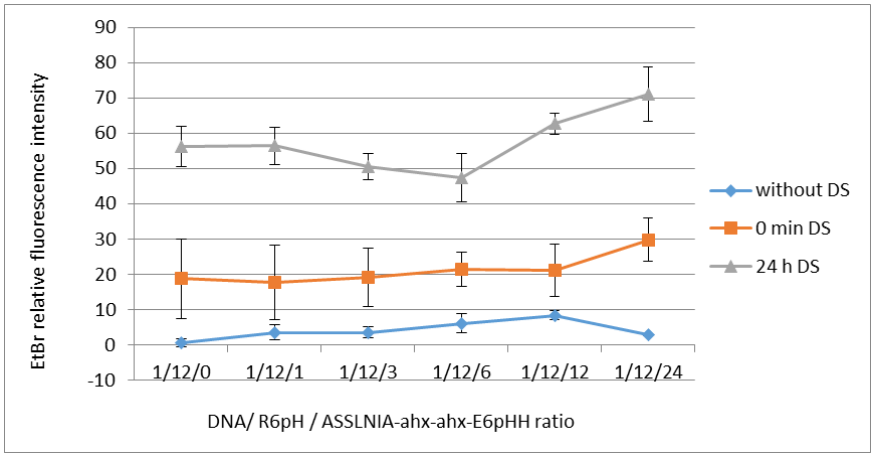

β-galactosidase activity after DNA delivery using peptide carriers at various ratios of DNA / carrier1 / carrier2. The mean and standard error of the mean are shown.

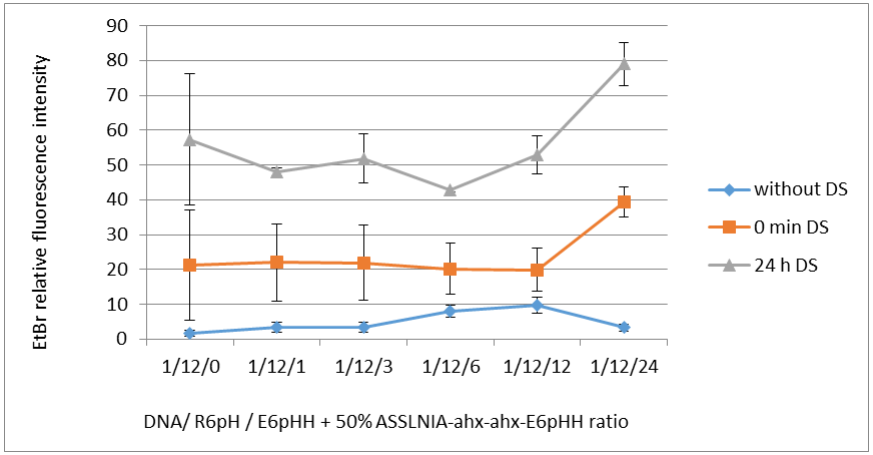
# Results of transfection of plasmid DNA with the *GFP* gene after inclusion of the ASSLNIA ligand to the carrier composition

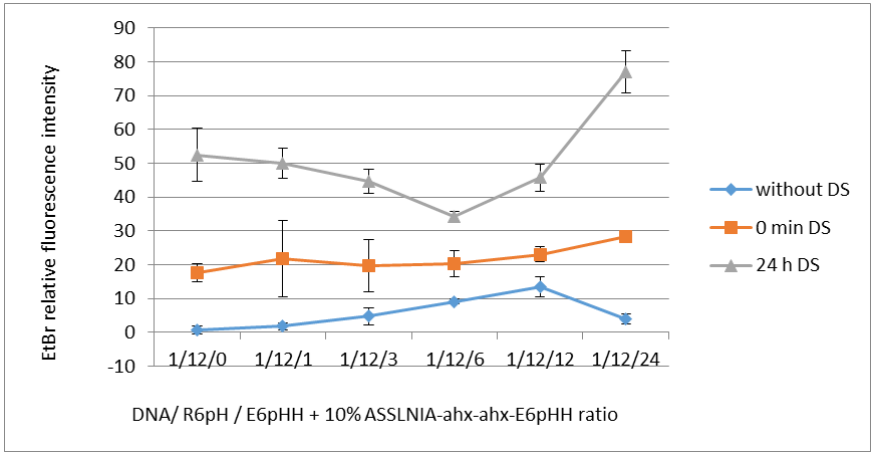


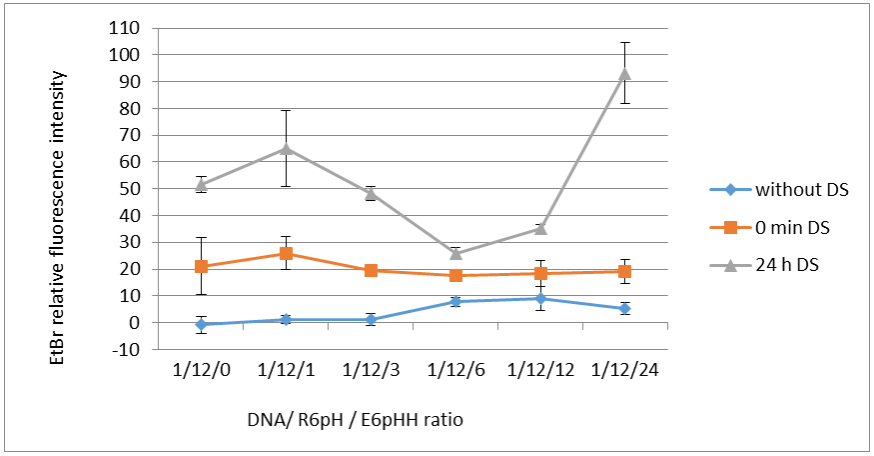

The number of cells expressing *GFP* after delivery of plasmid DNA using peptide carriers. Charge ratio DNA / carrier1 / carrier2 = 1/12/6. The mean and standard error of the mean are shown.

# DNA compaction with peptide carriers



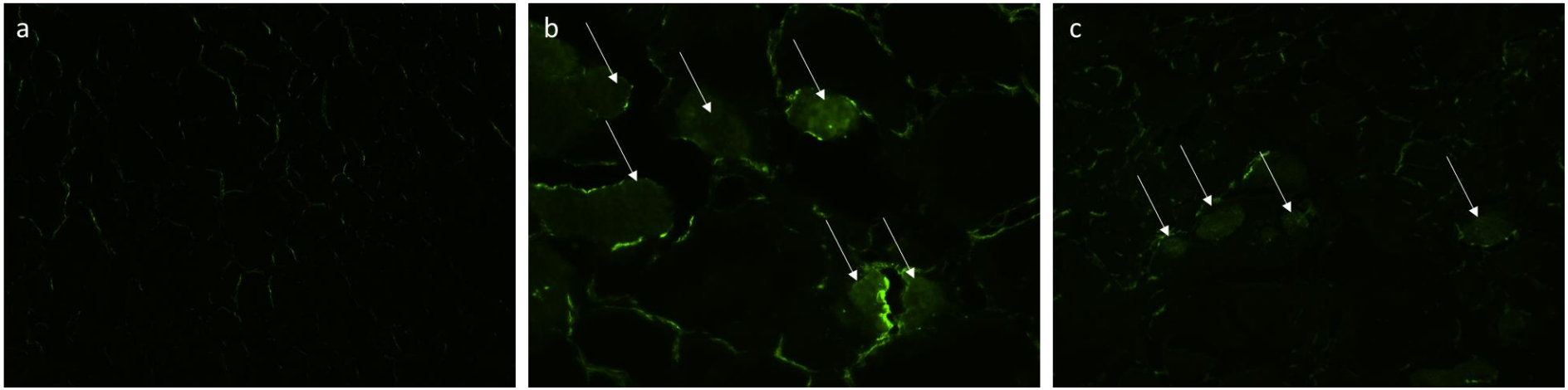



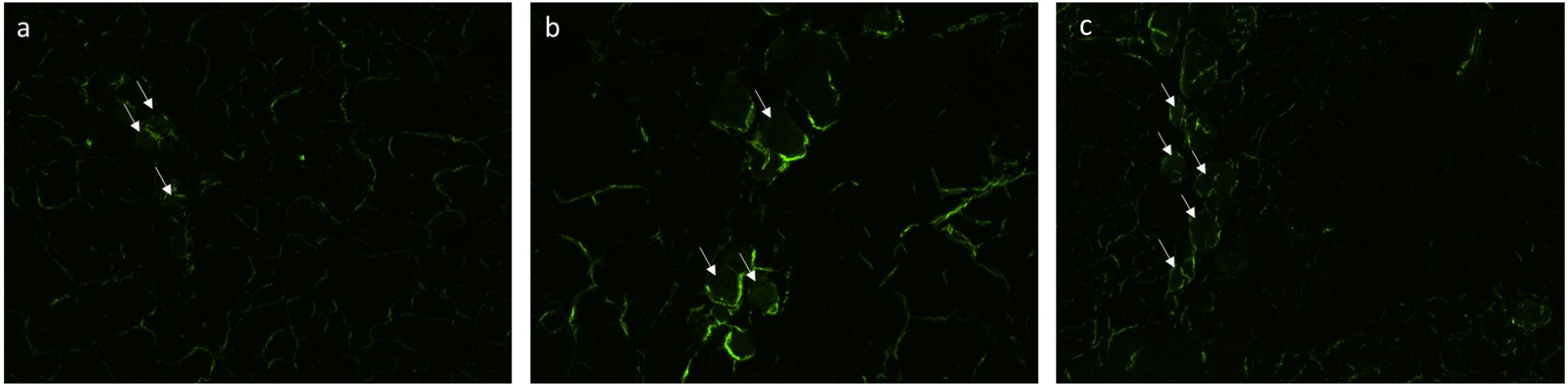

Relative intensity of EtBr fluorescence after DNA binding at different charge ratios of DNA / carrier1 / carrier2 before the addition of dextran sulfate (without DS), after the addition of DS (0 min DS), and 24 hours after the addition of DS (24 h DS). Standard deviation is indicated.

# The result of in vivo delivery of plasmid encoding *GFP* gene to the femoral muscle of mdx mice




Microphotographs of intact muscle tissue (a), muscle tissue after delivery of plasmid with the *GFP* gene using the commercial carrier TurboFect (b), as well as using the carrier R6pH / E6p with a charge ratio of DNA / carrier 1 / carrier 2 - 1/12/6 (c). Magnification x200.

# The result of in vivo delivery of plasmid encoding *GFP* gene to the femoral muscle of mdx mice




Microphotographs of muscle tissue after delivery of plasmid with the *GFP* gene using the R6pH / E6pH carrier at a DNA / carrier 1 / carrier 2 charge ratio of 1/12/12 (a), R6pH / E6pHH at a ratio of 1/12/6 (b), R6pHH / E6p at a ratio of 1/12/12 (c). Magnification x200.

**Conclusions:**

1. Most of the studied complexes are not toxic for C2C12 cells
2. The studied carriers are capable to efficiently deliver pDNA to C2C12 cells. The most effective one is the R6pH / E6pHH carrier at CR 1/12/6
3. The addition of 10 mol% of the ligand to the complexes formed with R6pH / E6pHH carrier formed at CR 1/12/6 leads to an increase in transfection efficiency
4. Most of the studied carriers can efficiently condense DNA and form complexes  prone to interaction with polyanions
5. After plasmid DNA delivery to muscle tissue, green areas were detected, indicating *GFP* gene expression of in vivo

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