Universität Rostock Traditio et Innovatio

Universitätsmedizin Rostock

A novel magnet-bead based vector system for microRNA delivery in cardiac regenerative medicine

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Introduction – Clinical background

Cardiovascular diseases are the leading cause (~25%) of death worldwide (1)

Problem:

No significant regeneration potential of adult cardiomyocytes

Damaged myocardial tissue has very limited capacity of self-renewal

Fig. 1: Donor heart (2)

Alternative therapeutic strategies?
→ Stem cells?

Eurotransplant Statistics - 2014

1140 - persons on the waiting list for donor hearts

615 - perforemd heart transplantations

(1) Center for disease control and prevention
(2) Die Welt (2013)

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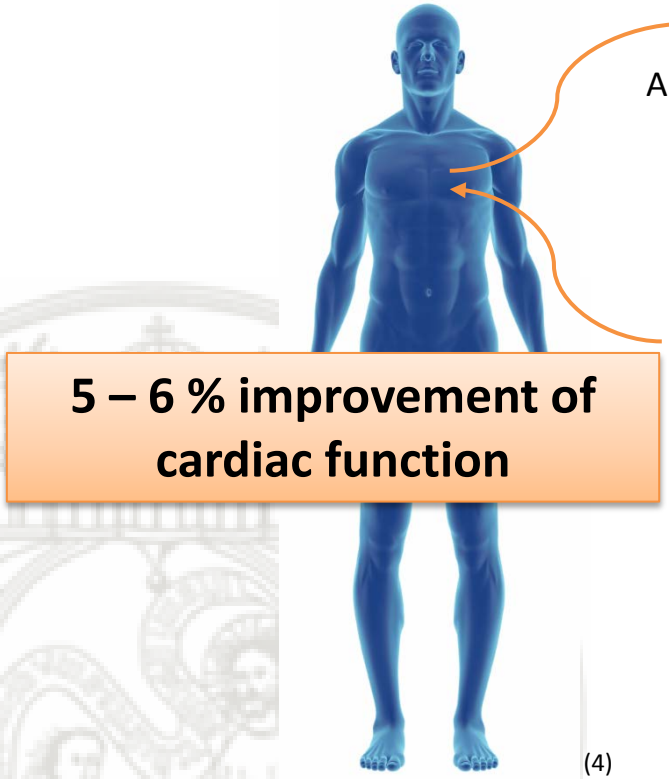
Introduction – Cardiac stem cell therapy

PERFECT – Phase III clinical trial in Rostock (3)

- Transplantation of CD133$^+$ stem cells during coronary artery bypass grafting (CABG)



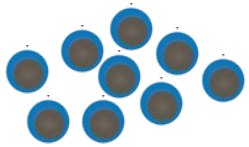
(4)

5 – 6 % improvement of cardiac function

Aspirations of sternal bone marrow

(5)

Isolation of CD133$^+$ stem cells



Intramyocardial CD133$^+$ stem cell transplantation
+
CABG

(3) Donndoraf *et al., Trials* (2012) (ClinicalTrials.gov Identifier: NCT00950274)
(4) Jeffrey *et al., Procedia Computer Science* (2015)
(5) CliniMACS Prodigy® System from Miltenyi

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Introduction – Cardiac stem cell therapy

Problems:

Low cell amounts from patients

Low stem cell retention (5 - 10%) (6-8)

Solutions:

Amplification of positive stem cell effects by genetic modification

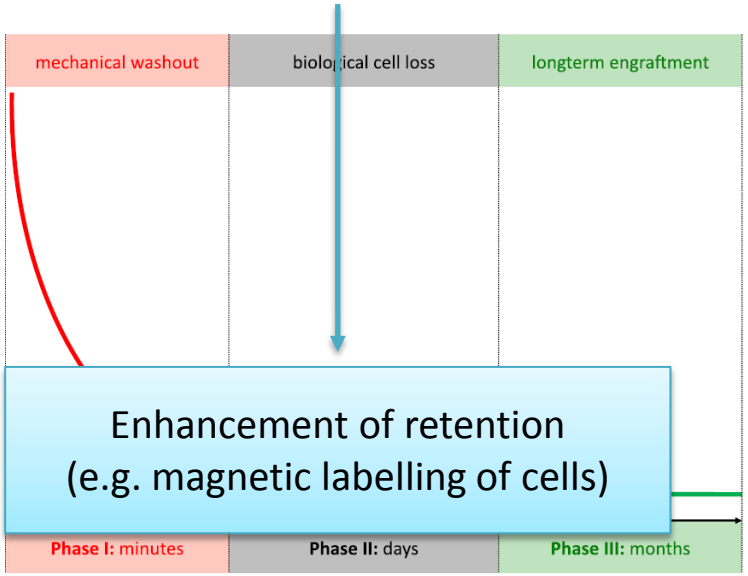

Fig. 2: Fate and survival of an intracardial cell transplant. (9)

(6) Goussetis *et al., Stem Cells* (2006)
(7) Caveliers *et al., Q J Nucl Med Mol Imaging.* (2007)
(8) Musialek *et al., J Nucl Cardiol.* (2011)
(9) Lang *et al., Eur J Nucl Med Mol Imaging.* (2014)

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Introduction – Vector system

1. miRNA-mediated cell modification
   - Transient modification
   - Multiple effects: (10-13)
     - Paracrine - mediated angiogenesis
     - Differentiation
     - Cell survival
     - Proliferation

2. Magnet- bead based vector system
   - Cell targeting
   - Cell retention

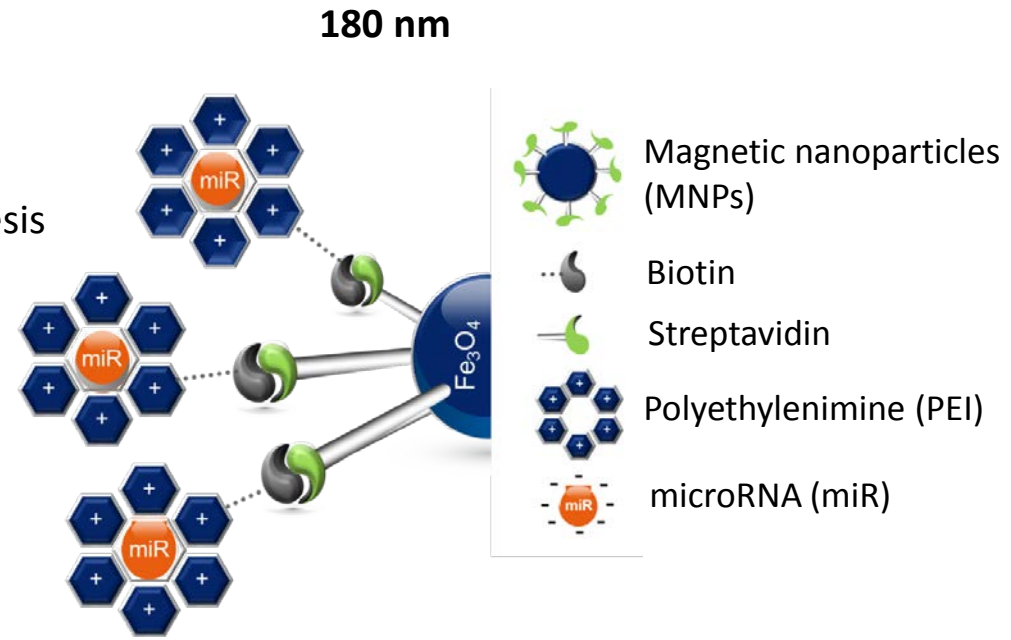

Fig. 3: Schematic structure of paramagnetic miR/PEI/MNP vector system. (15)

(10) Wen *et al.*, *PLaS One* (2014)
(11) Zou *et al.*, *Neural Regen Res.* (2014)
(12) Xu *et al.*, *Sci China Life Sci.* (2014)
(13) Kim *et al.*, *Cell Death Dis.* (2014)
(14) Schade *et al., Int J Mol Sci. (2013)*

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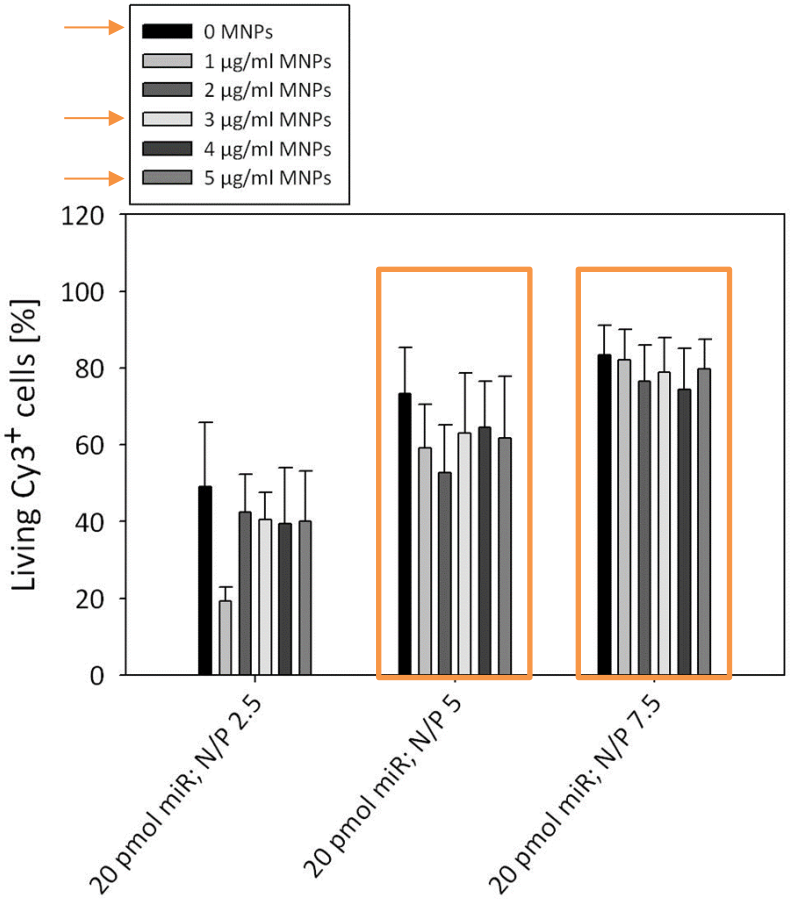
Results – Uptake efficiency

~ 80% Uptake efficiency



$$\text{N/P ratio} = \frac{\text{Molar ratios of N in PEI}}{\text{P of nucleic acids}}$$

Fig. 4: Transfection optimization of CD133⁺ cells. Cells were transfected with Cy3-labelled complexes consisting of 20 pmol miR, three different N/P ratios (2,5; 5 and 7,5) and five different MNP amounts (0; 1; 2; 3; 4 and 5 µg/ml). Uptake efficiency was measured 18 h after transfection by flow cytometry. Values are presented as mean ± SEM; n = 4; ∗ = p ≤ 0.05.

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Results – Viability and stem cell marker expression

Comparable viability and stem cell marker expression

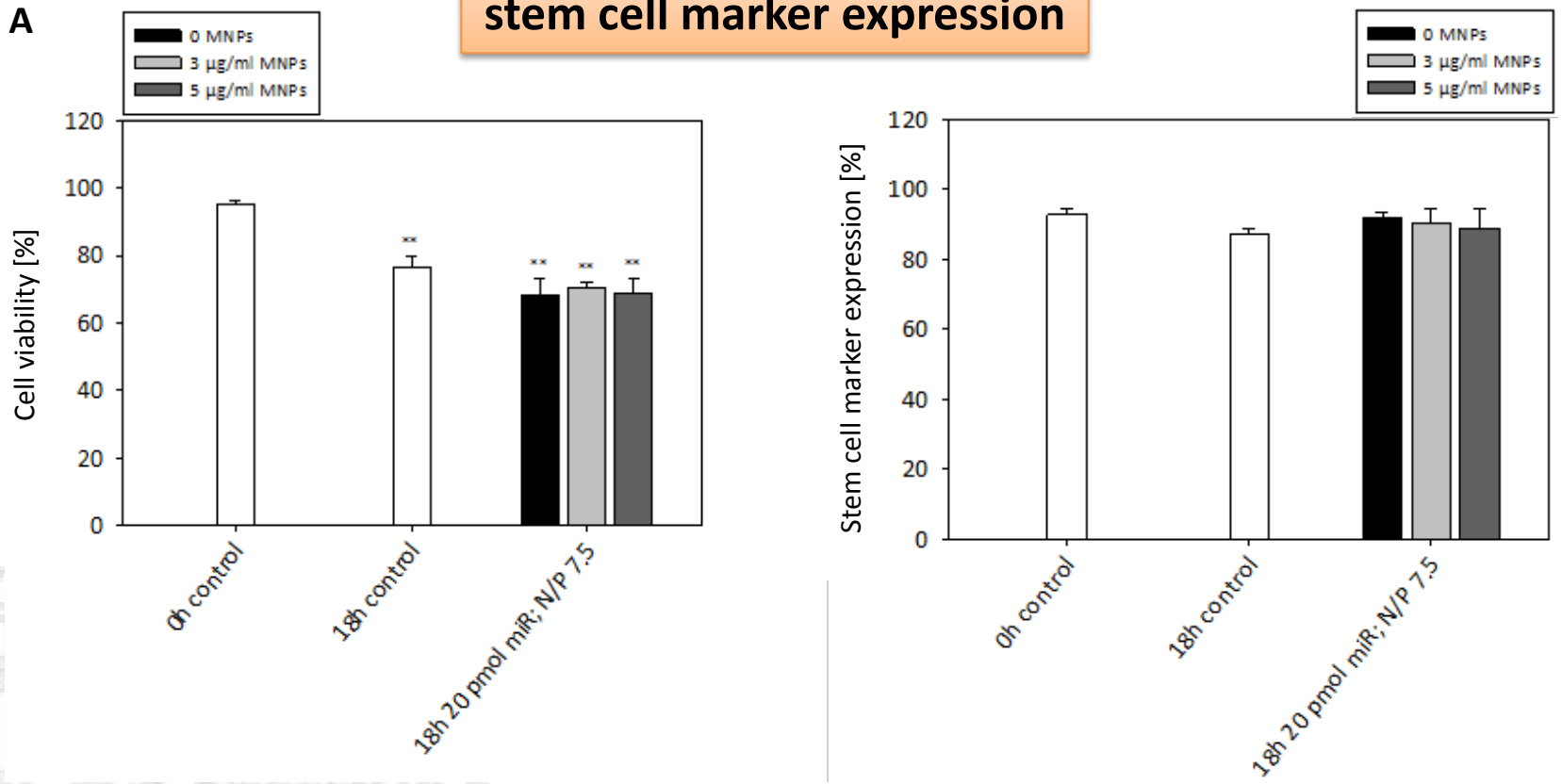

Fig. 5: Viability and purity of cells after transfection. Cells were transfected with optimized miR/PEI and miR/PEI/MNP complexes. Viability **(A)** and purity **(B)** were measured 18 h after transfection by flow cytometry. Untransfected cells 0 h and 18 h after isolation were used as control. Values are presented as mean ± SEM; n = 3; ∗ = p ≤ 0.05; ∗∗ = p ≤ 0.01; ∗∗∗ = p ≤ 0.001.

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Results – Magnetic targeting

Cells can be magnetically guided

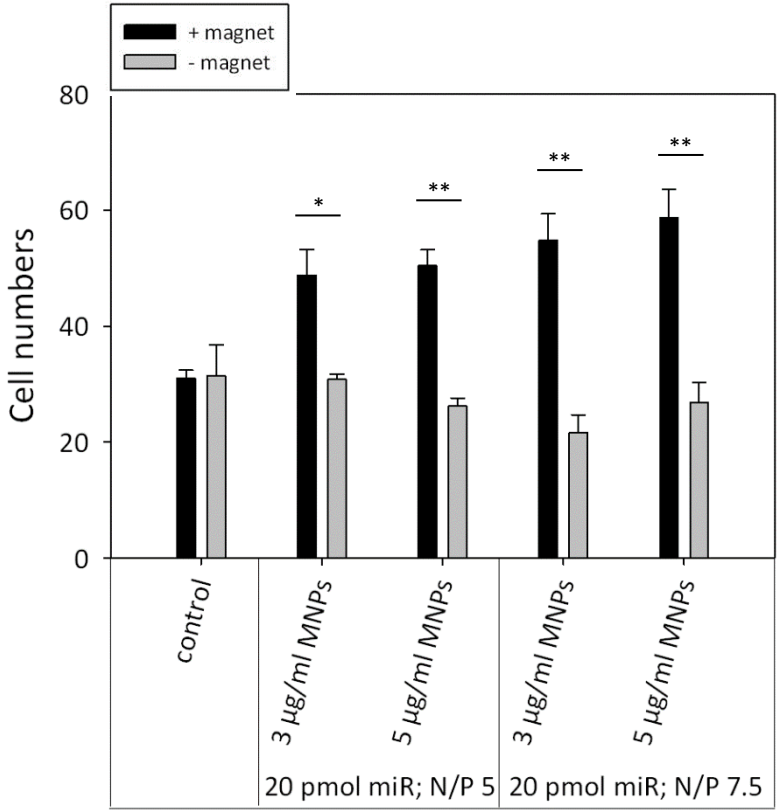

Fig. 6: Magnetic targeting of transfected CD133⁺ cells. Cells were transfected with optimized complexes. 18 h after transfection a magnetic field was applied for 24 h. Cell numbers of both areas (with and without magnet) were counted. Untransfected cells were used as control. Values are presented as mean ± SEM; n = 3; ∗ = $p \leq 0.05$; ∗∗ = $p \leq 0.01$; ∗∗∗ = $p \leq 0.001$.

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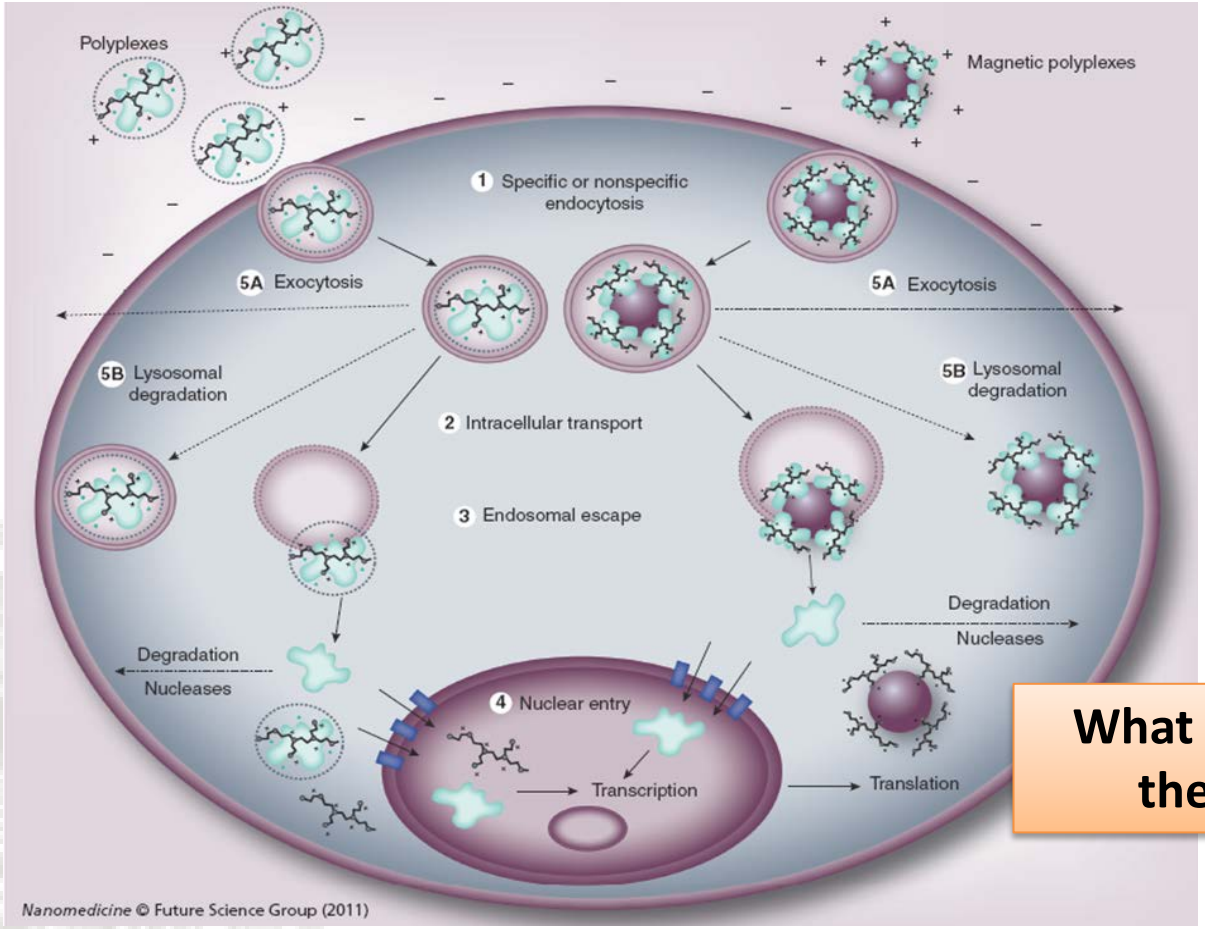
Results – Intracellular pathway



What happens to the vector?

Fig.7: Comparison between intracellular pathways of polyplexes and magnetic polyplexes.
(Delyagina *et al.*, *Nanomedicine(2011*)

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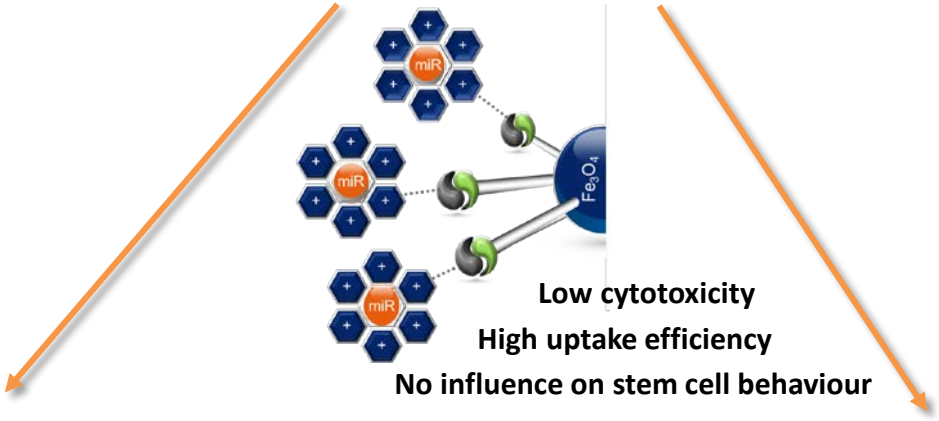
Perspective: clinical use of genetically modified magnetic CD133+ stem cells



Supportive stem cell effects

Improvement of:

- Cell survival
- Cell proliferation
- Angiogenesis
- Cell retention & targeting

Tissue replacement

- Programming into specific cell types

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Thank you!

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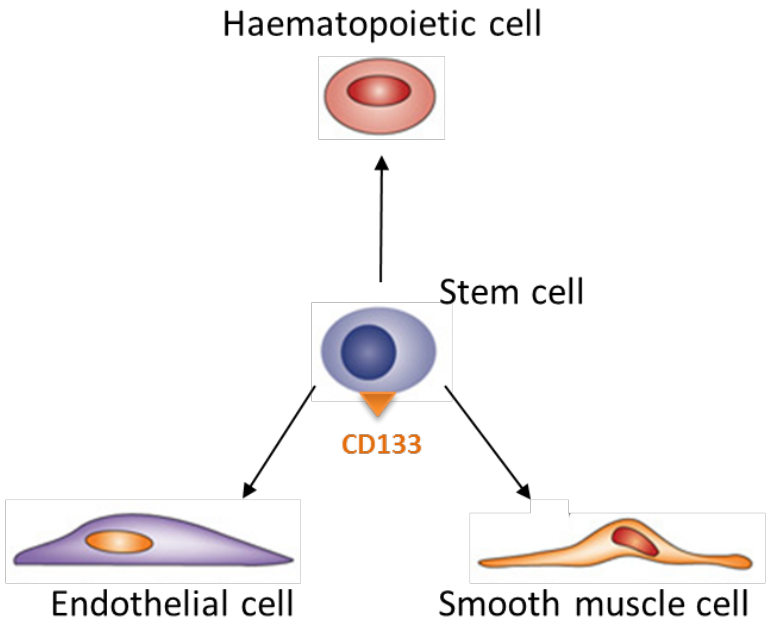
Introduction – CD133^{+} stem cells

Therapeutic tool in tissue regeneration

- CD133 antigen (Prominin-1) is a transmembrane protein
- Multipotent differentiation potential



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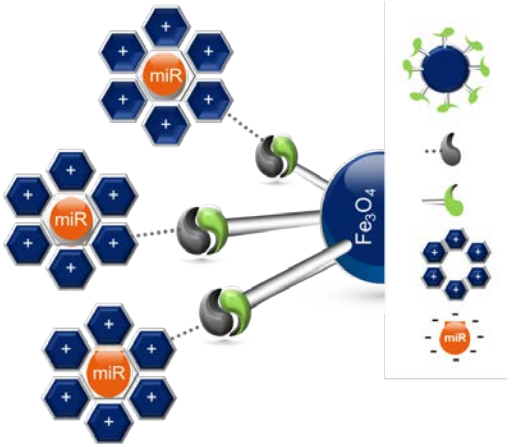
N/P ratio

Definition:

$$\text{N/P ratio} = \frac{\text{molar ratios of nitrogen residues in PEI}}{\text{phospahet of nucleic acids}}$$



Calculation:

$$N/P\ ratio = \frac{c(PEI)[mM] * V(PEI)[\mu l]}{n(miR)[pmol] * 0.12}$$

c = concentration
V = volume
n = amount of substance

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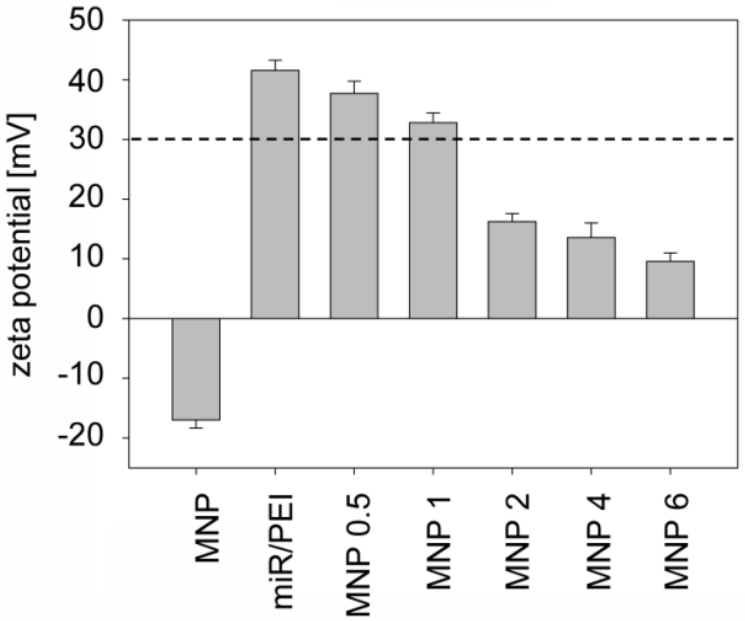
Characterization of transfection complexes



	MNP	miR/PEI	MNP 0.5	MNP 1	MNP 2	MNP 4	MNP 6
Particle size, nm ± SD	98.1 ± 5.4	99.4 ± 7.9	108.9 ± 10.4	169.9 ± 15.9	178.1 ± 19.1	175.9 ± 16.8	201.9 ± 15.6

Fig: Surface charge and particle size of MNPs and transfection complexes were determined by DLS and PALS. Zeta potential data are presented as mean ± standard error, *n* = 10. Particle size data are presented as mean ± standard deviation, *n* = 10.
(Schade *et al*., 2013 in *Int J Mol Sci*.)

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miR condensation assay

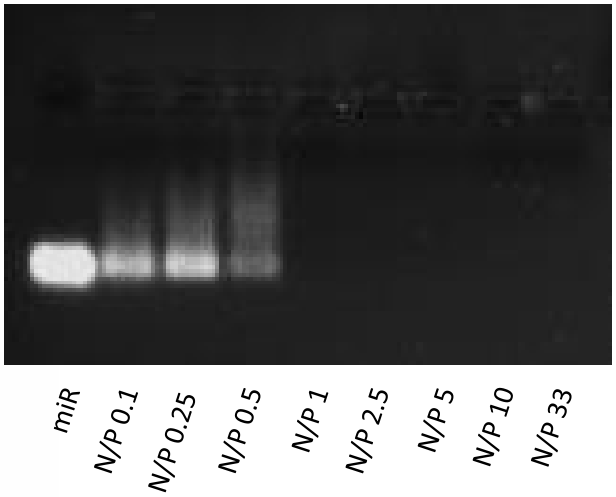

Figure: Formation of miR/PEI complexes at different N/P ratios detected by gel electrophoresis. Unbound miR was stained with ethidium bromide.

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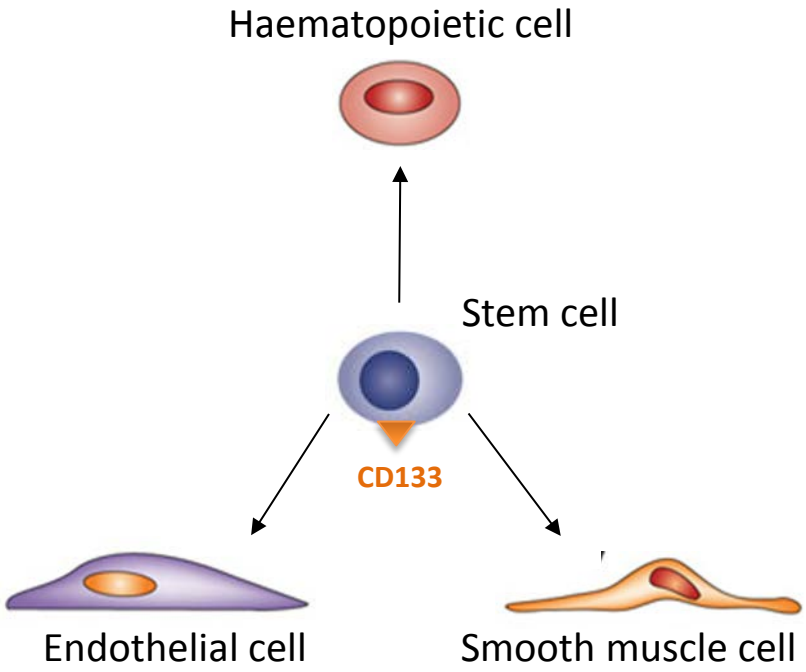
Haematopoietic cell
Stem cell
CD133
Endothelial cell
Smooth muscle cell

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