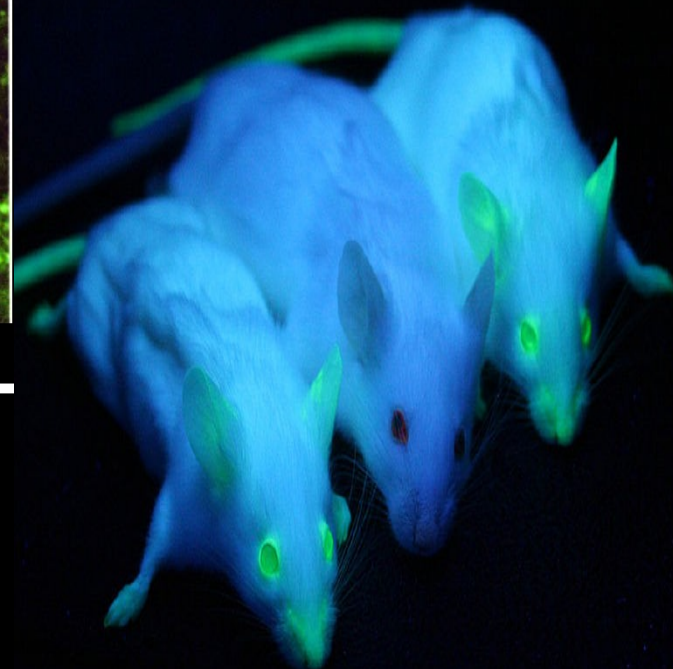
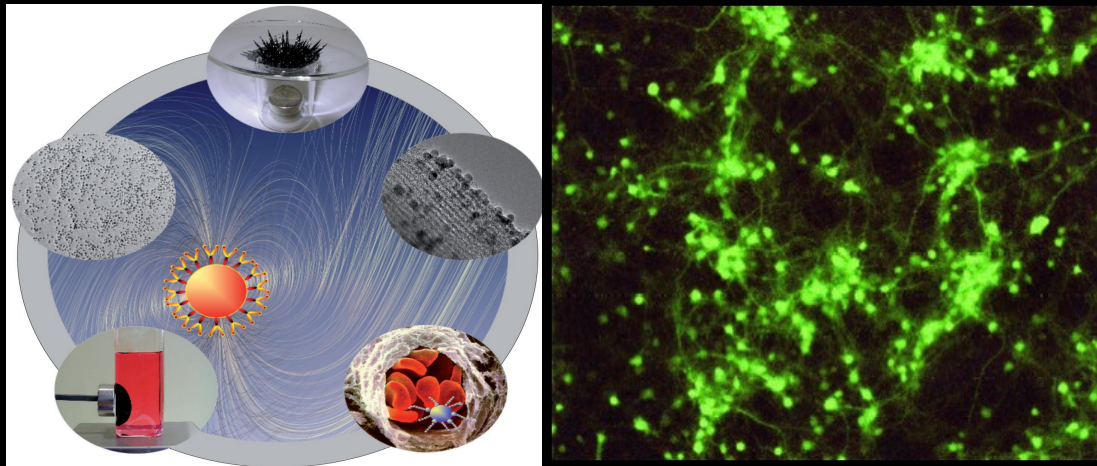


# SYNTHESIS OF IRON OXIDE NANOPARTICLES FUNCTIONALIZED WITH POLYETHYLENIMINE (PEI) FOR DNA BINDING AND GENE DELIVERY



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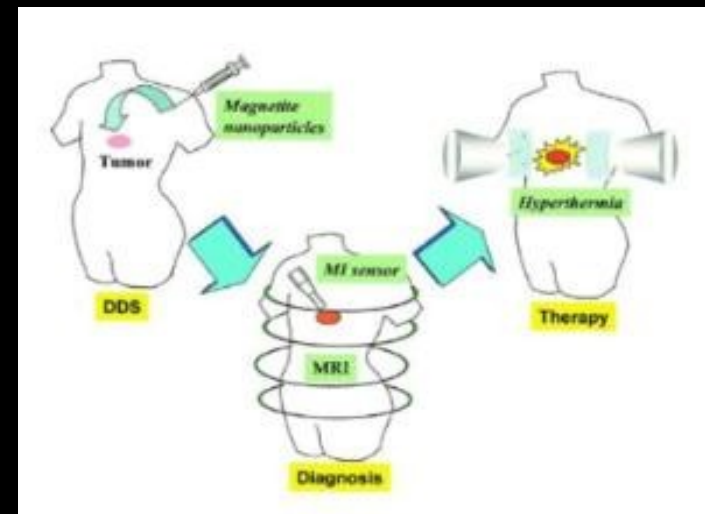
# Objectives:

Develop a fast and efficient method for **delivery of genes to target cells** for the purpose of ;

- Over expression of introduced genes
- On/off regulation of endogenous genes
- Gene repair

# Introduction

- Paramagnetic iron oxide nanoparticles have been widely used in numerous applications like;
  - i. MRI (magnetic resonance imaging)
  - ii. Tissue repair
  - iii. Hyperthermia
  - iv. Drug delivery
  - v. DNA transfection



- What is transfection?
  - ✓ Transfection is process by which nucleic acids are introduced to eukaryotic cells using non viral vectors
- Why genes are transfected to cells?
  - ✓ Mainly because of the resulting therapeutic benefit for the individual

# Types of vectors

```
graph TD; A[Types of vectors] --> B[Viral]; A --> C[Non viral];
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## Viral

### Gene transduction

- Retroviruses
- Adenoviruses etc.

## Non viral

### Gene transfection

- Plasmids
- Liposomes

- Viral vectors show high gene transfer efficiency, but are deficient in several areas, including the **induction of a host inflammatory and immune response.**
- Some of these problems can be circumvented by employing non-viral vehicles, such as cationic liposomes or polymers.

- However non-viral transfection has a lower efficiency when compared with the viral transfection. That is because cellular uptake mainly depends on,
  - Size of DNA
  - Charge of DNA
  - Stability of DNA

# Main modes of non-viral gene delivery

Polypeplexes (Polymeric delivery systems)

Lipoplexes (Liposomal delivery systems)

# Liposomes

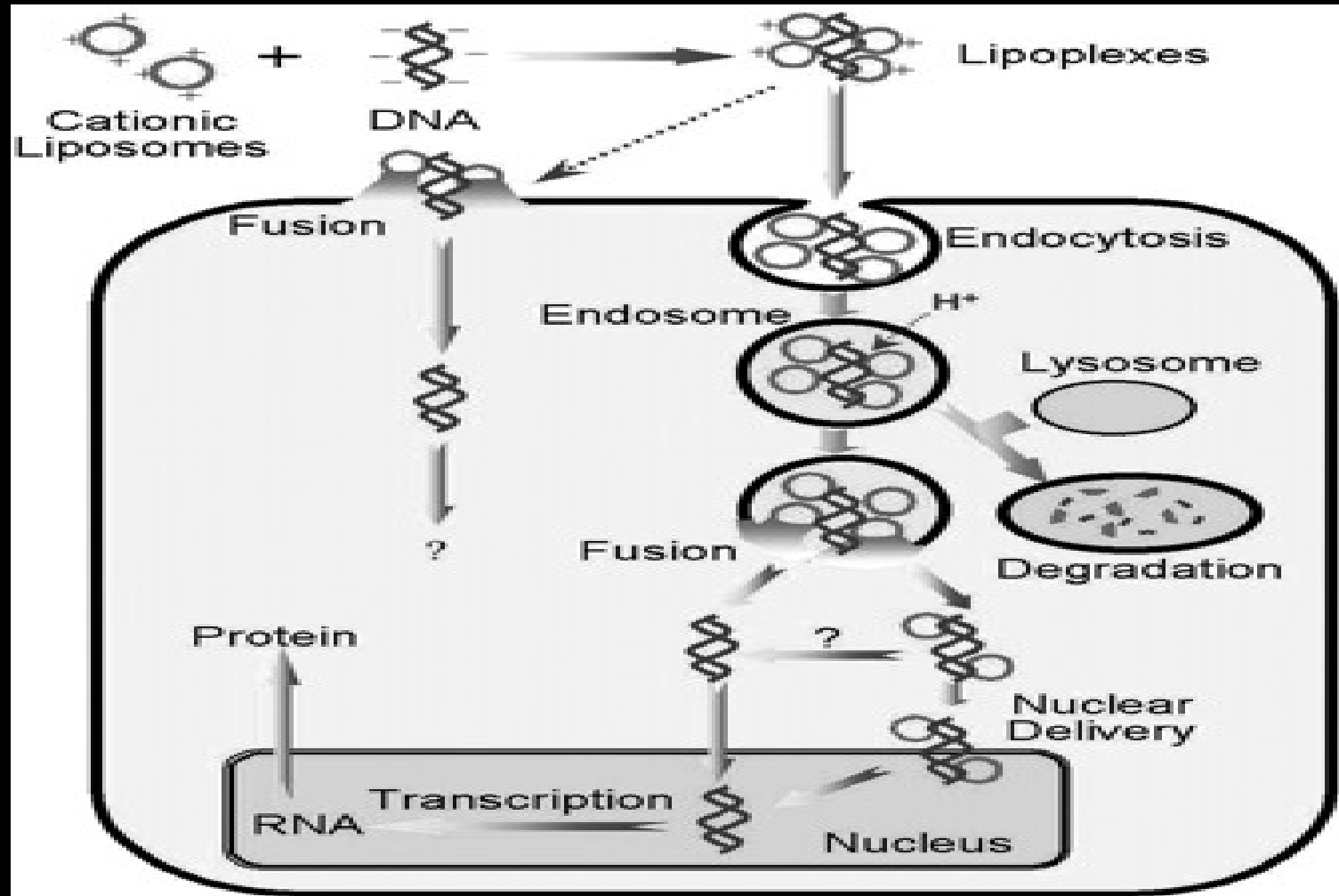
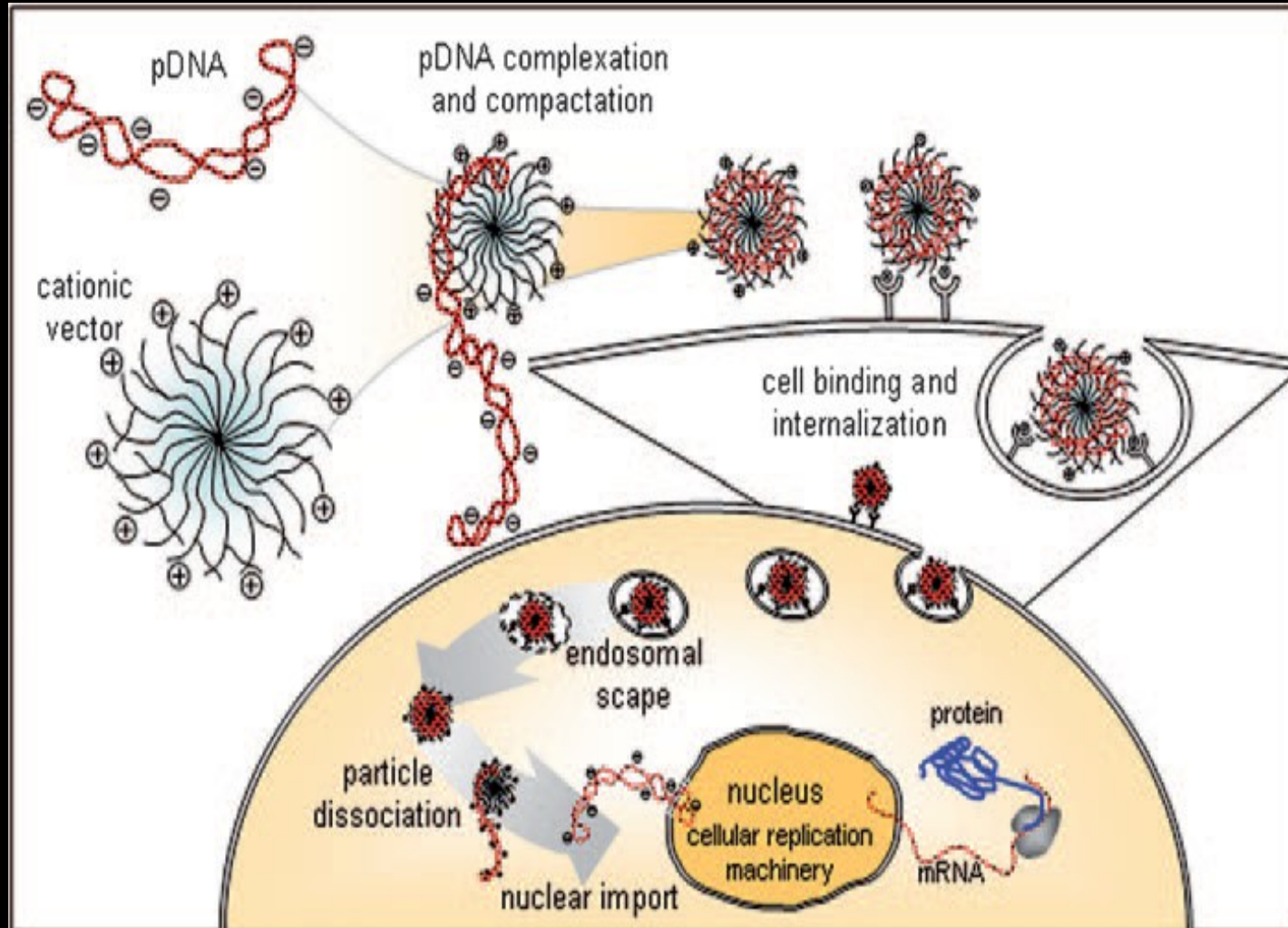


Fig.1 Entry of DNA/ Liposomes in to cells

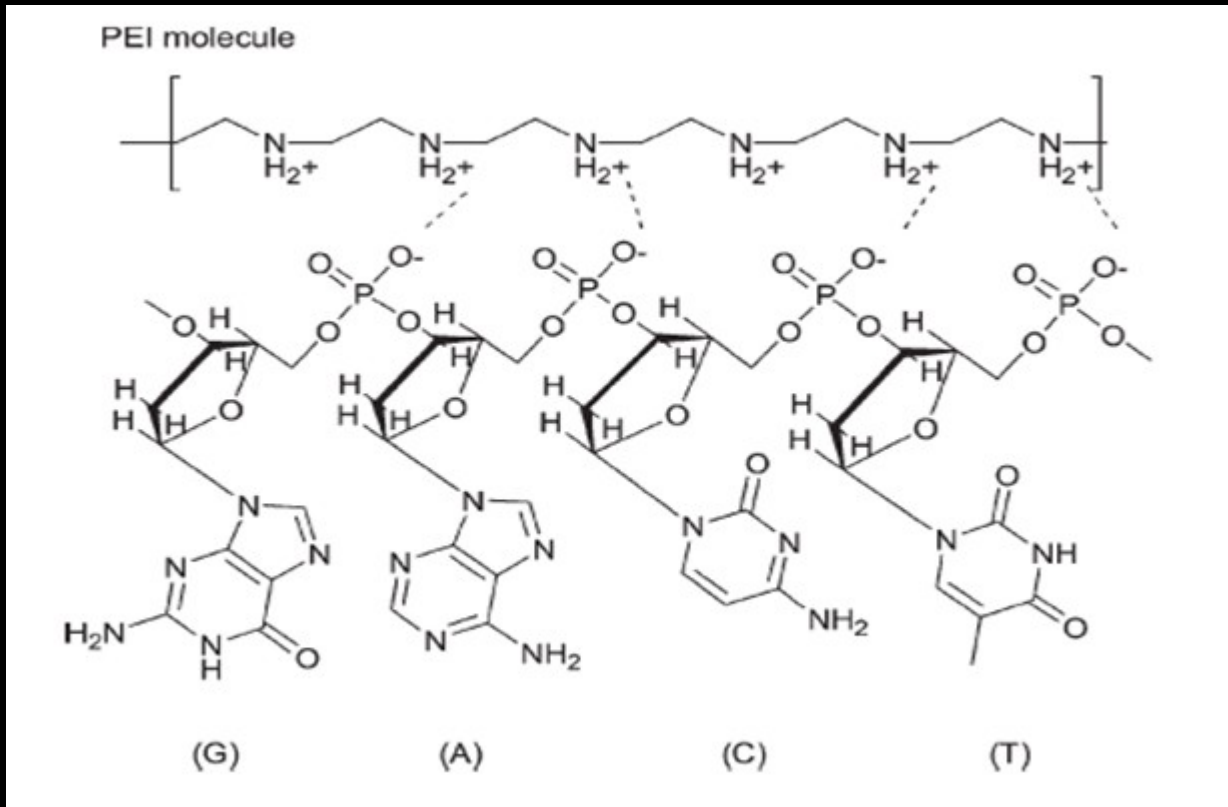
# Polyplexes



**Fig.2 Entry of non- viral polyplexes to the cells**

- **Why use Polyethyleneimine?**
  - ✓ PEI protects RNA and DNA from degradation by enzymes.
  - ✓ PEI has ability to complex and condense DNA into stable complexes.
  - ✓ PEI has the highest DNA binding capacity.
  - ✓ PEI easily associate with IONPs.
  - ✓ PEI apparently induces massive proton accumulation in the endosomes followed by passive chloride influx leading to osmotic swelling (Proton sponge effect).

# Polyethyleneimine



**Fig.3 Electrostatic interaction between DNA and PEI**

# History

1. In 2000- AAV( adeno associated virus) delivered to cells by Cathryn and co-workers (viral)
2. In 2002- Use of PEI coated magnetic nanoparticles for gene delivery by Scherer *et al* (size was ~200 nm)
3. In 2007- Covalent coupling of PEI to iron oxide composite via glutardialdehyde linker by McBain *et al* (size was 200- 300 nm)
4. In 2008- Petri- Fint *et al* work on synthesis of PEI coated IONP (involves dialysis for 2 days, too lengthy !!!)

5. In 2009- Wang and his co- workers have synthesized PEI coated IONP in the presence of citric acid (involves lot of neutralization processes and dialysis for 48 hours, also particle size 200-800nm)

**But we.....**

- Synthesized IONP within 25- 30 minutes
- PEI coating, just took 30 minutes
- Size was around 3 nm
- Simple and a rapid method



# Methodology:

- Co-precipitation was the method which we used during our study.
- Iron oxide nanoparticles were synthesized from aq  $\text{Fe}^{2+} / \text{Fe}^{3+}$  (1:2 ratio) salt solutions by the addition of ammonia under  $\text{N}_2$  atmosphere in room temperature.



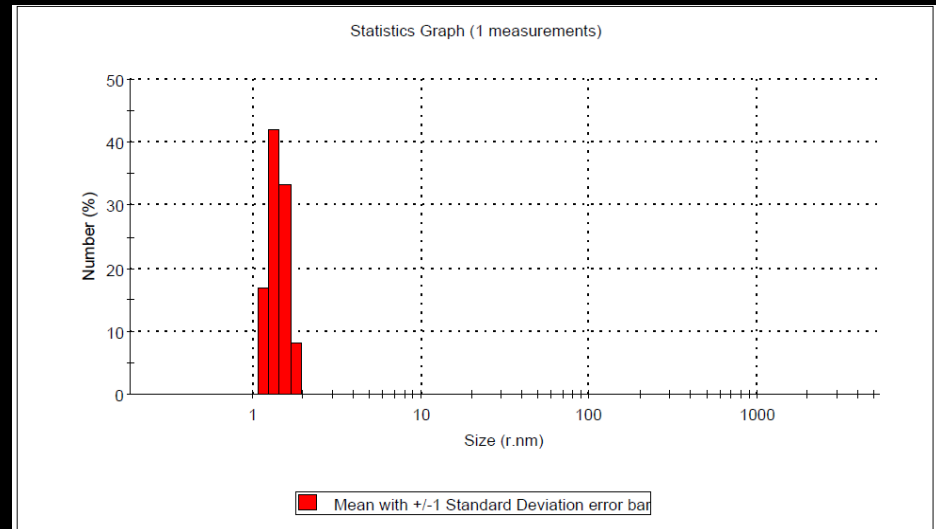
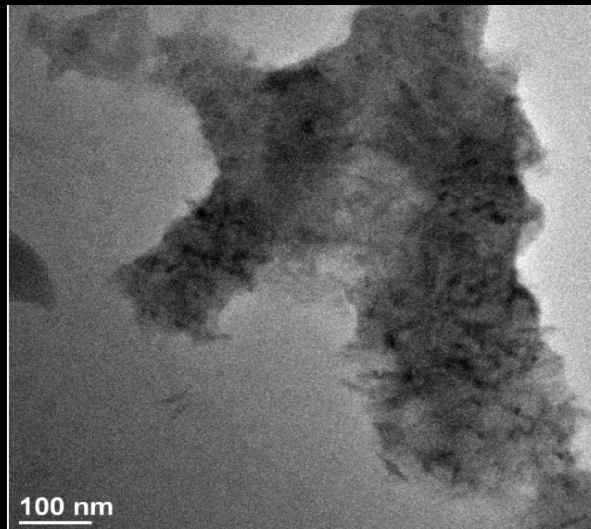
**Fig.4 Synthetic set up of magnetic nanoparticles and magnetic attraction**

# Functionalization with PEI

- Three forms of PEI mainly used.
  - i. 22 kDa- linear
  - ii. 25kDa- branched
  - iii. 50kDa- branched
- During our study we used 25kDa branched polymer.
- Functionalization was carried out in a basic medium using 20%(w/w) PEI solution for 30 minutes at 50 °C

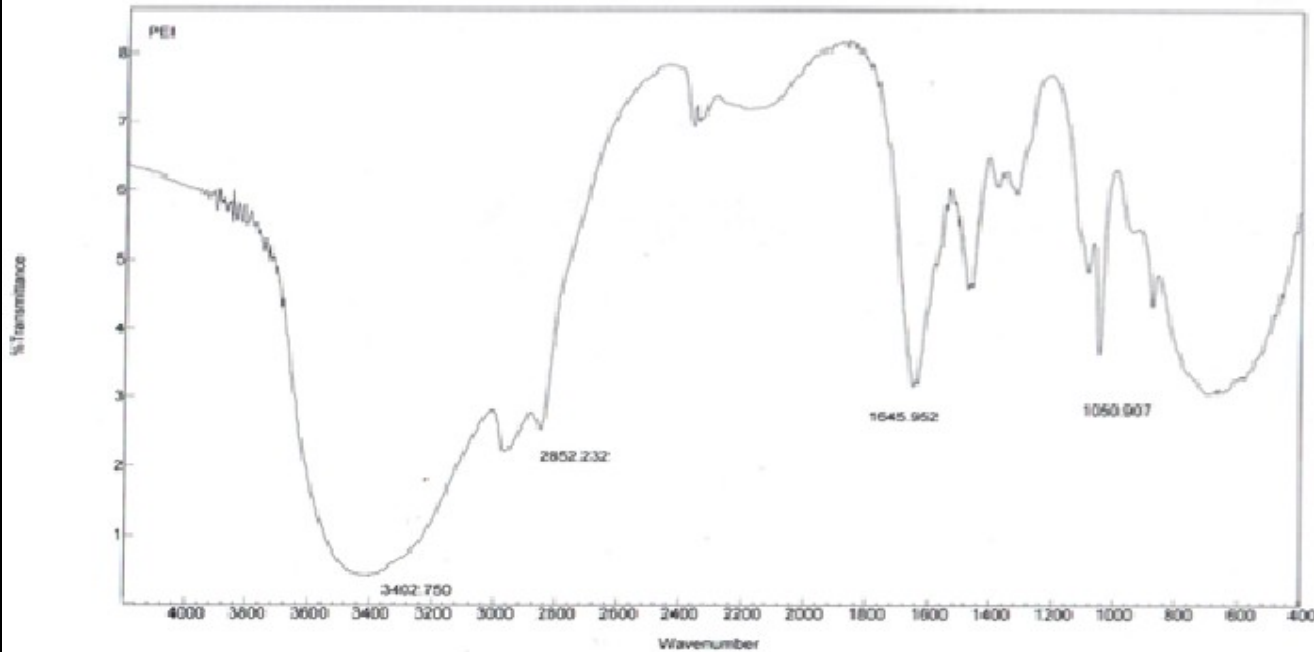
# Results and discussion:

- The size of PEI functionalized IONP was characterized using TEM and particle analyzer.
- Particle analyzer gave the size around 3 nm.

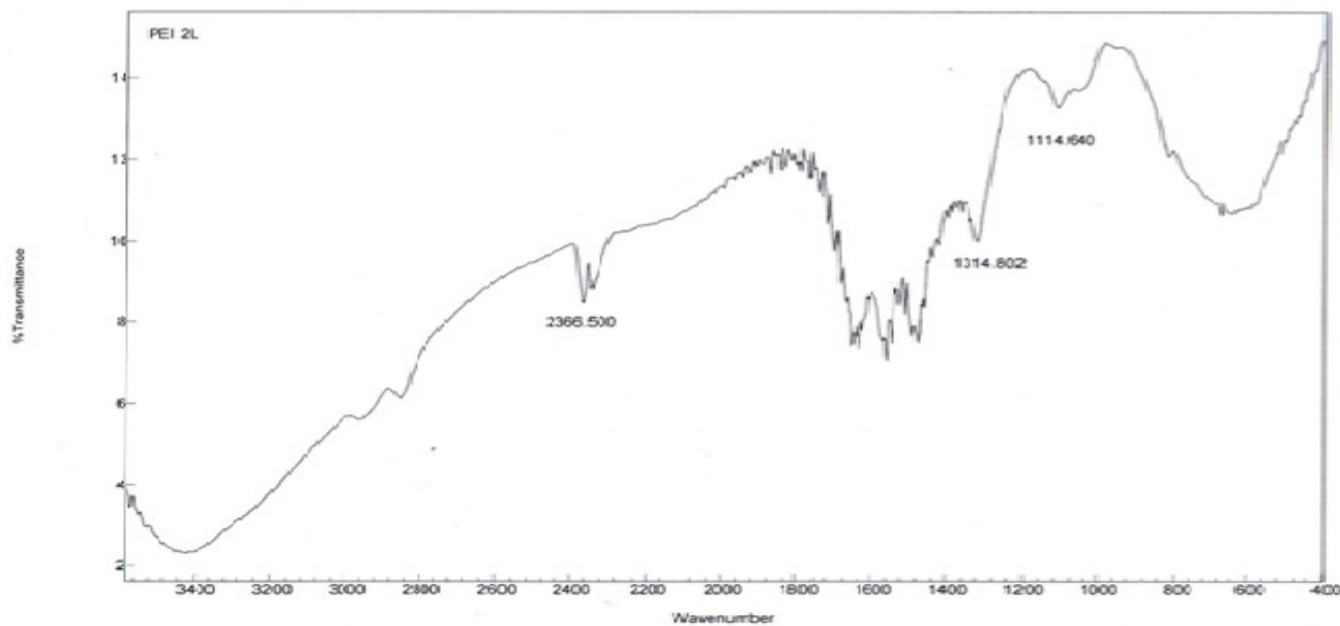


**Fig.5 TEM image and histogram obtained from particle analyzer**

- This is because of particle agglomeration, where each iron oxide nanoparticle is associated with more than one strand of PEI and each strand is attached to more than one nanoparticle which causes **bridging aggregation**. This is in accordance with the results reported in the work done by Petri-Fink *et al* (Petri-Fink A, Steitz B, Finka A, Salaklang J. & Hofmann H. (2008). Effect of cell media on polymer coated superparamagnetic iron oxide nanoparticles (SPIONs): Colloidal stability, cytotoxicity, and cellular uptake studies. *European Journal of Pharmaceutics and Biopharmaceutics* 68(01):132)

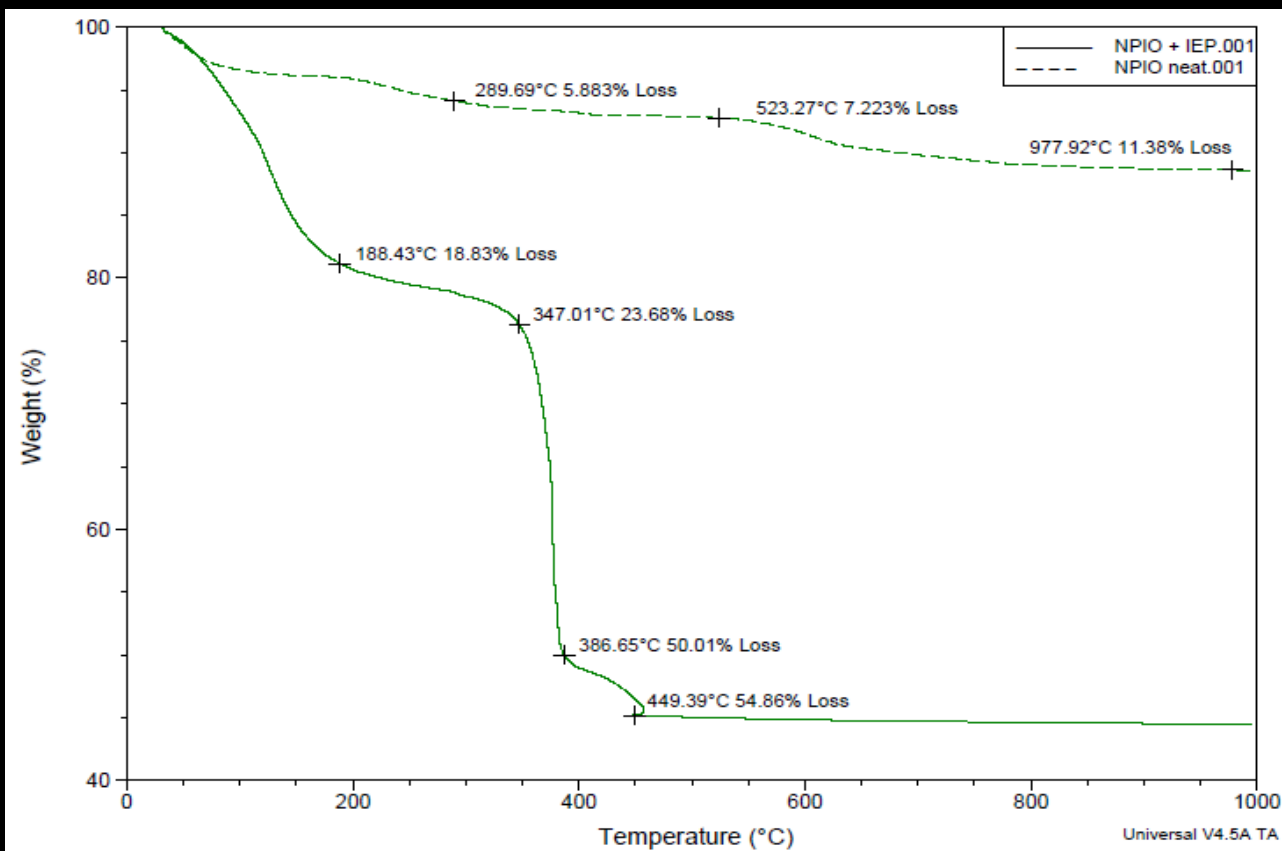


**Fig. 6 FT-IR spectrum of 20% (w/w) PEI**



**Fig. 7 FT-IR spectrum of PEI coated IONP**

- TGA curves confirmed that PEI is coated on IONP
- Weight loss of neat IONP= 11.38%
- Weight loss of PEI coated IONP= 54.86%

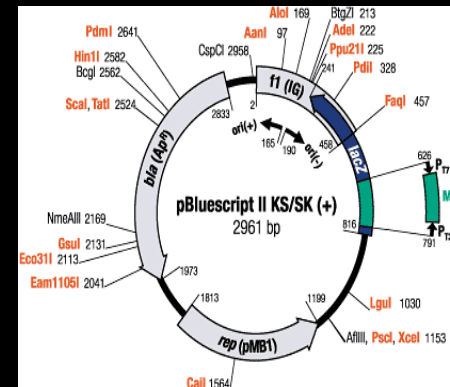
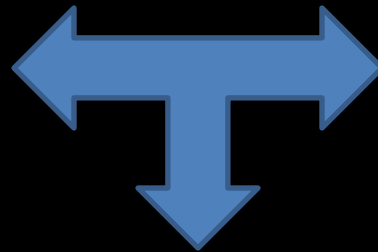


**Fig.8**  
**Comparison**  
**of TGA**  
**curves of**  
**neat IONP**  
**and PEI**  
**coated IONP**

# Application of PEI functionalized IONP for gene delivery

1. DNA binding assessment
2. Magnetofection of HEp2 cells

# DNA binding assessment

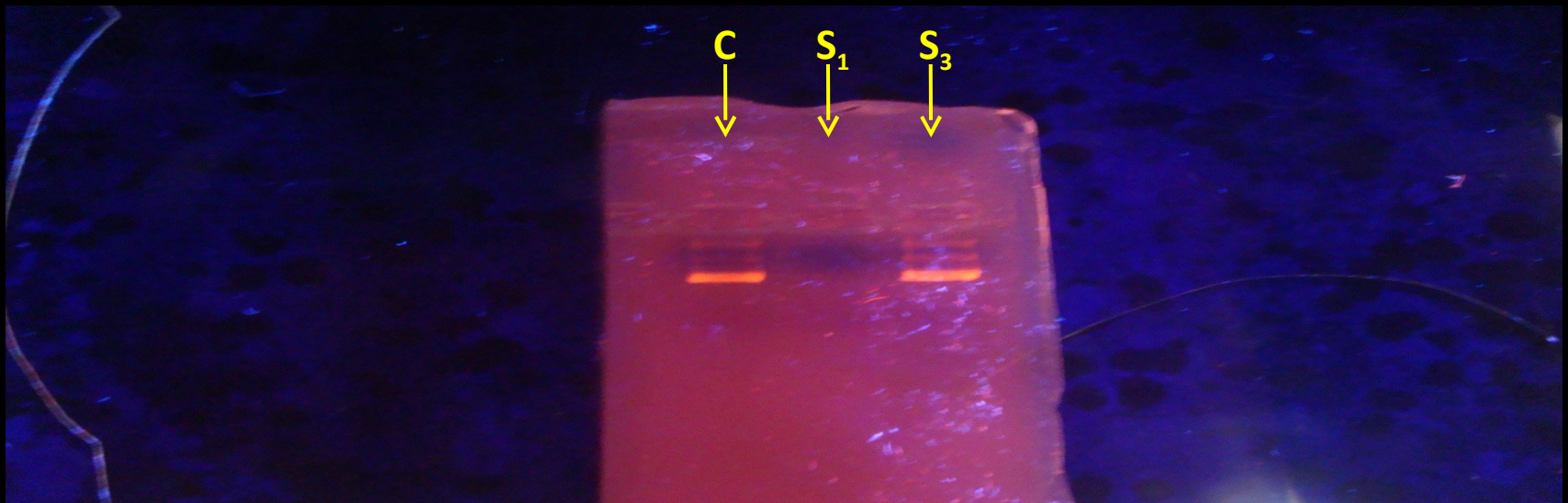


PEI coated IONP

pBS vector

- pBS vector and PEI coated IONP were incubated at room temperature.
- DNA complexed particles were collected by magnetic separation using a NdFeB magnet

- DNA bound on PEI coated IONPs was eluted using a buffer containing Tris, NaCl at 60°C
- Gel electrophoresis was carried to confirm the elution of DNA from nanoparticles



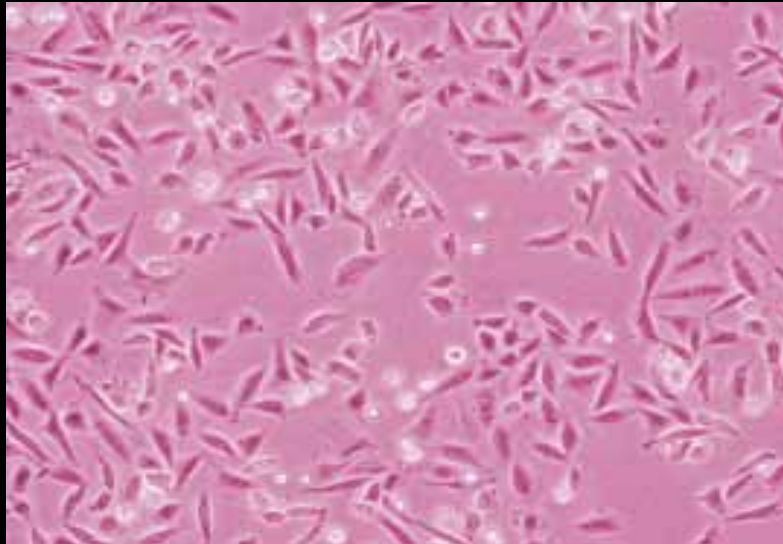
**Fig.9 Elution done at 60 °C,  
C- Marker, S<sub>1</sub> - Unbound DNA left in the solution, S<sub>3</sub> - Eluted  
DNA**

# Magnetofection

- During the course of our study PEI functionalized IONP were used to deliver the gene encoding for GFP (green fluorescence protein) to HEp2 cells
- pcDNA 3.1 contained the gene coding for GFP

## **HEp2 cell line**

- A cell line derived from epidermal carcinoma of larynx/ voice box in humans( organ in neck)
- Kindly donated by Dr.Preethi Soysa of Department of Biochemistry and Molecular Biology of Faculty of Medicine, UOC



**Fig.10 HEp2 cells viewed under a phase contrast microscope**

- HEp2 cells were cultured in a MEM ( minimum essential medium) medium.
- Prior to transfection  $1 \times 10^5$  cells/well were seeded at 90% confluency.
- Cells were allowed to take pGFP plasmids for 15-20 minutes under the influence of the magnetic field.
- Medium was replaced after 20 minutes.



**Fig.11 Cell culture preparations**

**Fig.12 Magnetic transfection done by keeping the well plate on a magnetic box**

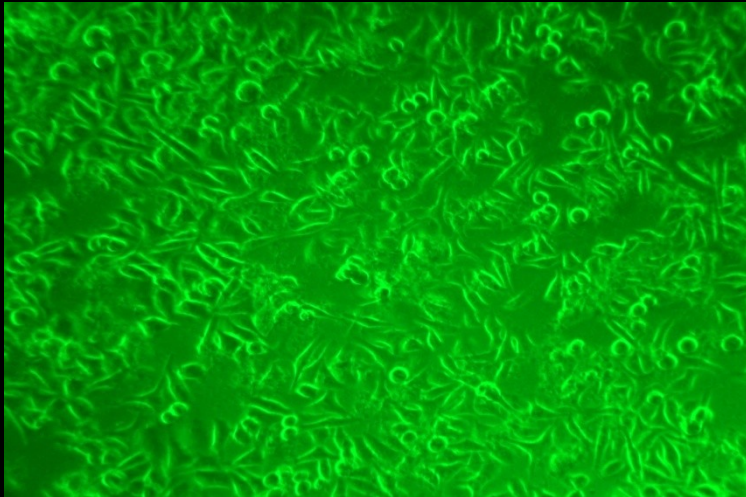


- After 24 hr incubation the cells were washed with PBS(phosphate buffered saline) and examine the expression of GFP by fluorescence microscopy.

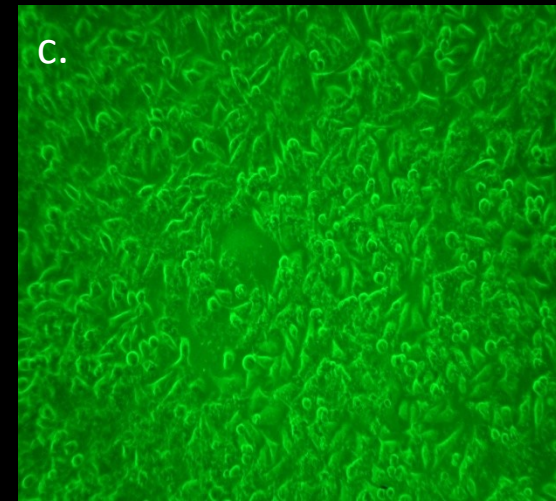
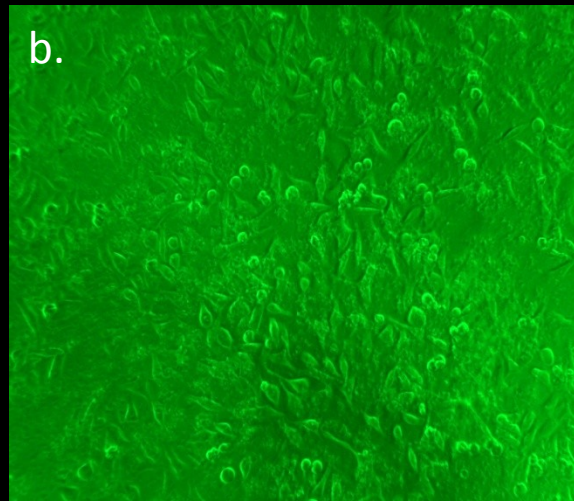
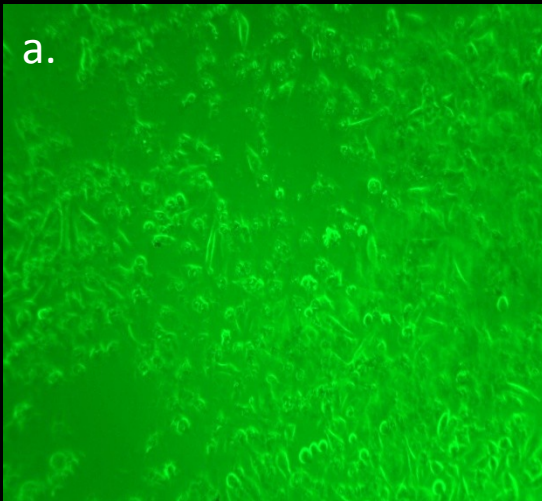


**Fig.13 Image capturing using fluorescence microscope**

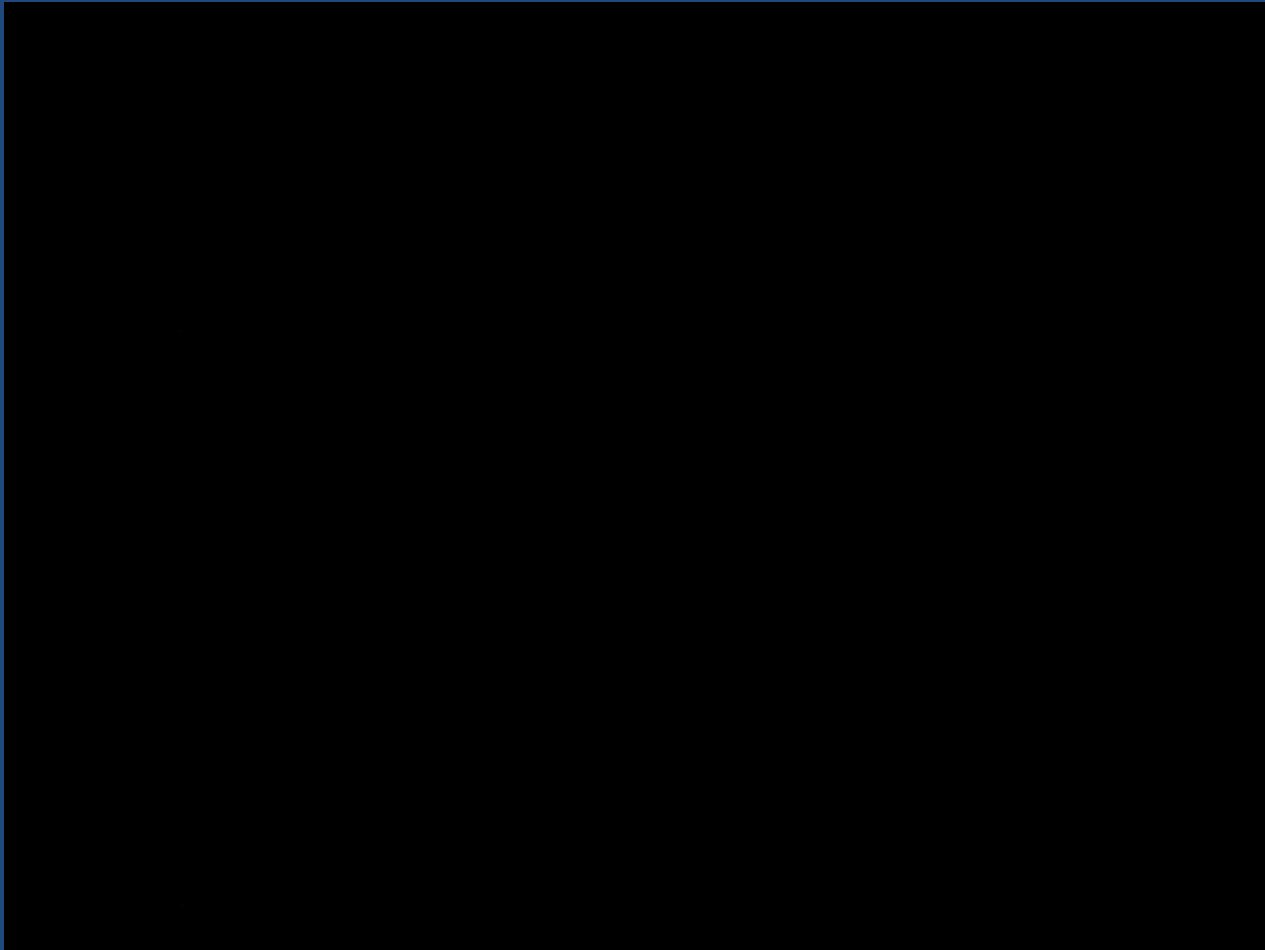




**Fig.14 cells with 90%  
confluency before  
transfection**



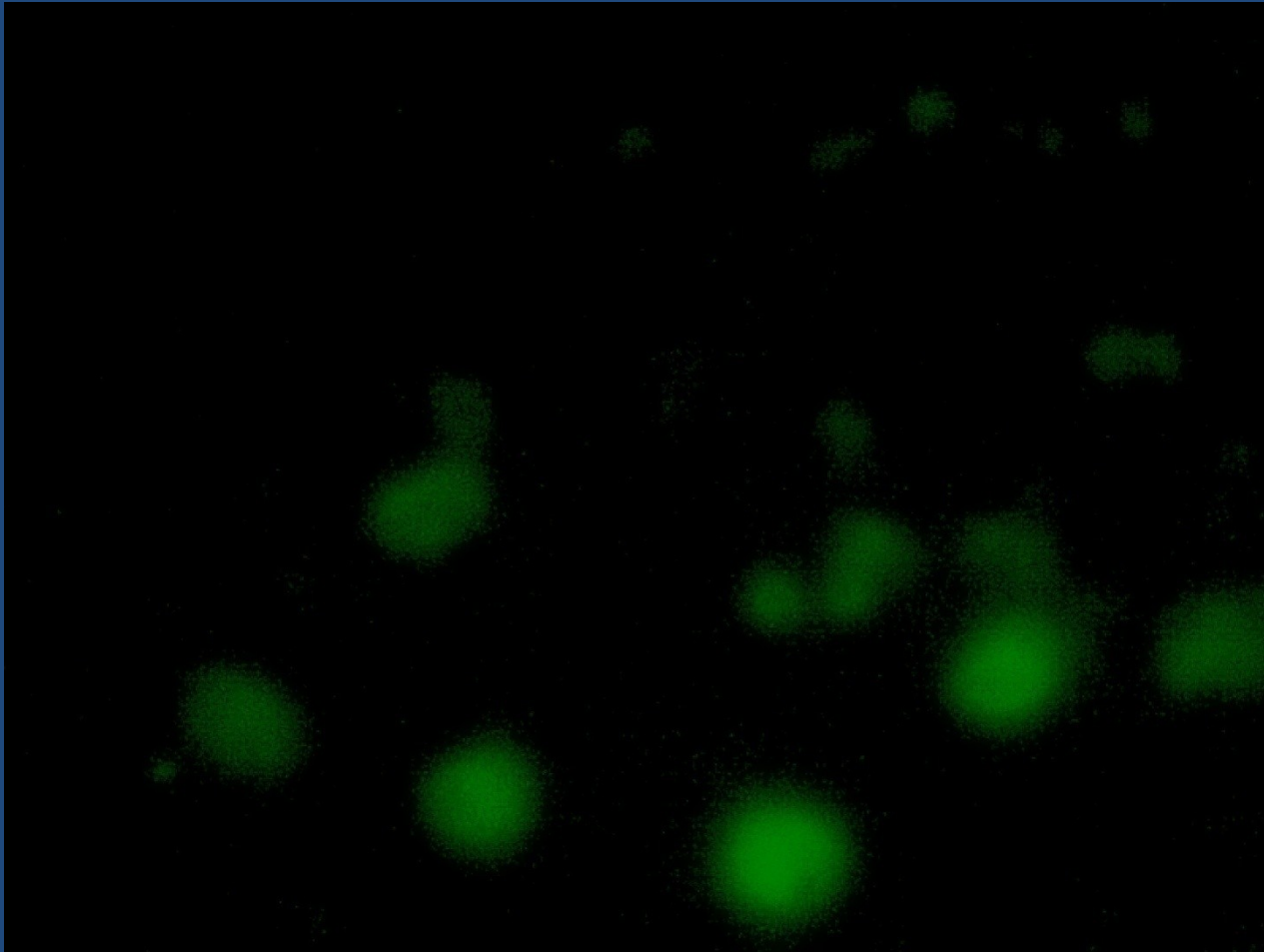
**Fig.15 a.) PEI + DNA , b.) PEI-IONP + DNA, c.) Untransfected  
cells**



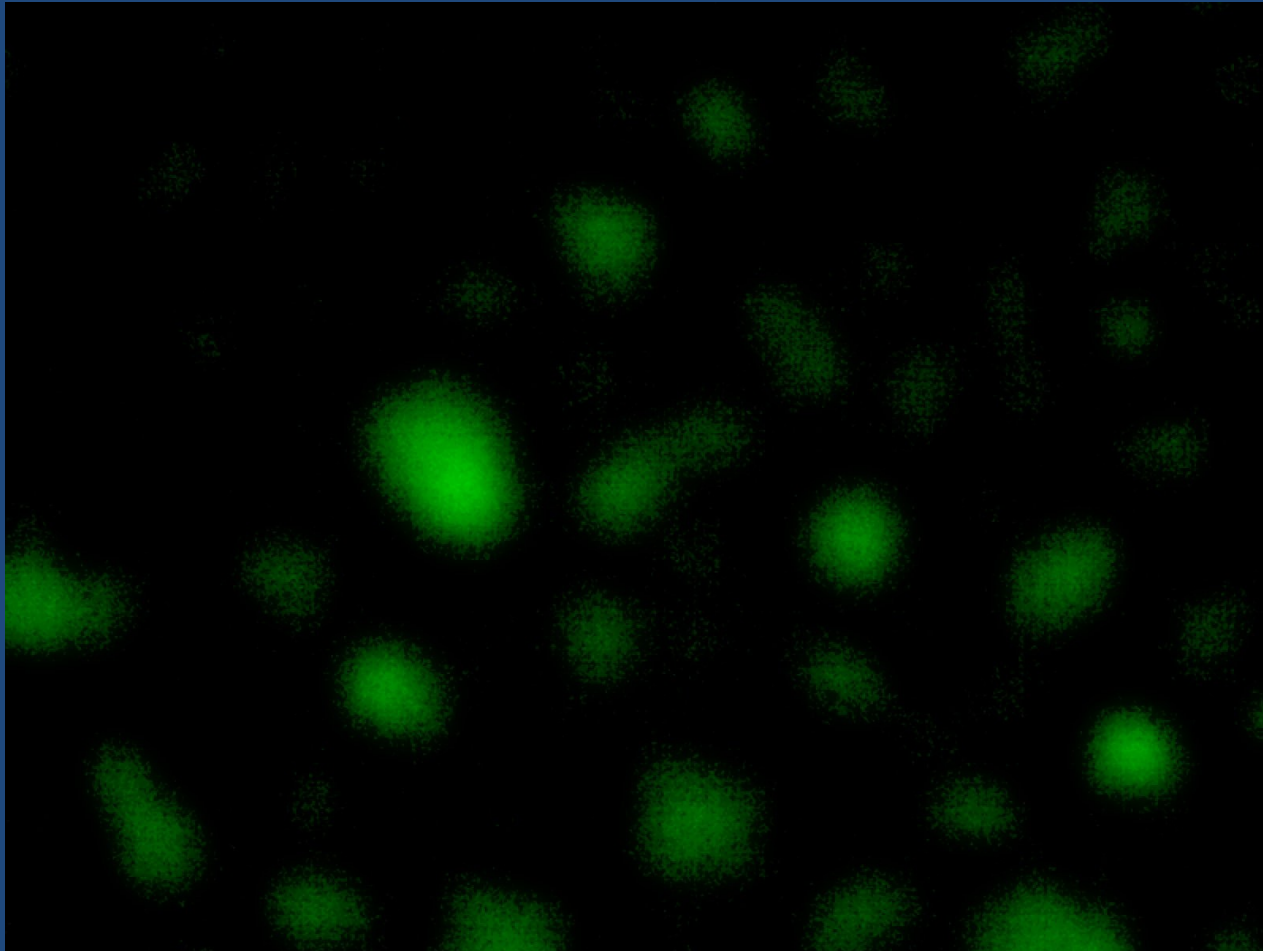
**Fig.16 No GFP expression in untransfected cells**



**Fig.17 GFP expression in cells transfected by PEI**



**Fig.18 GFP expression in cells transfected by PEI coated IONPs**



**Fig.19 GFP expression in cells transfected by PEI coated IONPS**

# Conclusion

- Drastically reduce the amount of PEI needed, since the particle size is very small
- There by toxicity is reduced
- Reduce the incubation time up to 15 minutes (Conventionally 4 hours)
- Simple method
- Easy to prepare

# Thank You !!!!!!!!

