Photochemical internalisation (PCI) – enhanced and site-directed mRNA delivery by light-induced endosomal release

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Background

- Nucleic acids are usually taken up into the cell by endocytosis, both if delivered as free molecules and if delivered by lipid- or polymer based delivery vehicles. Insufficient escape from endocytic vesicles often represents a significant barrier for efficient intracellular delivery and biological activity of various types of nucleic acids
- The Photochemical internalisation (PCI) technology can re-direct endocytosed molecules from endosomes to cytosol and can therefore be used to enhance intracellular delivery of nucleic acids
- Being a light-induced technology, PCI can target and enhance local mRNA delivery without increasing off-target effects

Technology and Results

PCI technology induces endosomal release - and enhances vehicle-mediated mRNA delivery *in vitro*

D Drug molecule
- mRNA

T The mRNA target
- Protein translation machinery

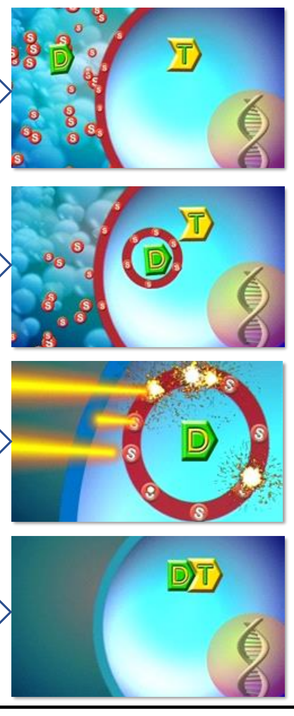
S The PCI component
- Fimaporfin ($TPCS_{2a}$) : Light sensitive amphiphilic molecule (photosensitiser)

STEP 1: Distribution
The photosensitiser (S, fimaporfin) and the mRNA molecules (D, drug) are injected into the body and meets the target cell. mRNAs may be naked or complexed with a delivery vehicle. Due to the amphiphilic nature of fimaporfin it inserts into the outside of the plasma membrane

STEP 2: Uptake
Fimaporfin and mRNA are endocytosed by the target cell. mRNA molecules will to a large degree be entrapped in endosomes unable to reach the protein translation machinery (T) in the cytosol. Fimaporfin is washed away from the cell surface, but will be retained on the inside of the endosomal membrane

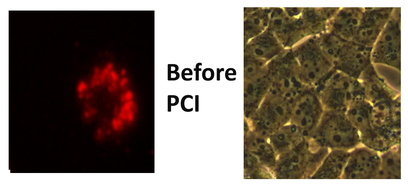
STEP 3: Light activation – endosomal release
Light activation of fimaporfin triggers generation of reactive oxygen species which affects the membrane integrity of the endosome, resulting in endosomal escape of the mRNA molecules into the cell cytosol

STEP 4: Hitting intracellular target – translation of mRNA
The mRNA meets the translation machinery in the cell cytosol and can be translated into a therapeutic protein



PCI can be used both with RNA complexed with delivery vehicles and with naked RNA molecules

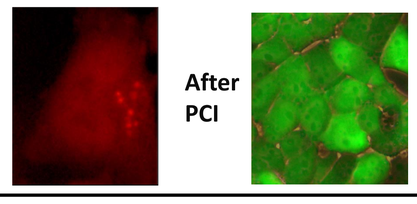
In vitro, PCI strongly enhances cytosolic RNA (siRNA and mRNA) delivery with several types of delivery vehicles.



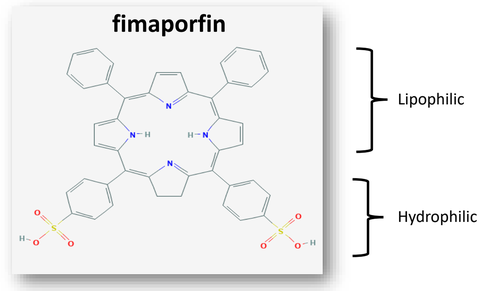
Before PCI

Labelled RNA molecules (PEI vehicle) in endosomes released into cytosol by illumination

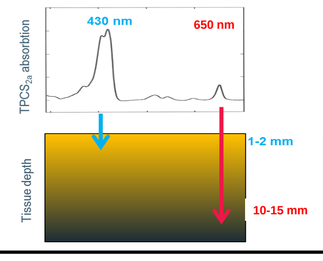
PCI-mediated endosomal release strongly enhances expression of GFP-encoding mRNA (PEI vehicle)



After PCI



- Easily produced
- Very stable (can be autoclaved, stable at room temperature for several years
- Can be mixed directly with naked RNA molecules and with most types of delivery vehicles
- Safety an tolerability demonstrated in humans (i.v. and i.d. administration)
- Light activation at λ_{max} = 420 nm (blue) and 652 nm (red)

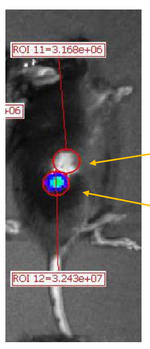


Depending on the desired treatment depth, illumination effects can be chosen to be shallow (blue light; 1-2 mm) or deeper (red light; 10-15 mm)

In vivo, PCI technology enhances delivery of naked mRNA to tumours, skin and muscle – no off-target expression or cytokine induction observed

> 30 times improvement of intratumoural mRNA delivery in two different tumour models

MC38 mouse colon cancer model - PCI/naked mRNA compared to LNPs

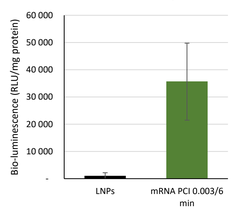


LNPs:
- MC3:DSPC:Chol:DMG-PEG at
- mol% composition of 50:38.5:10:1.5;
- 20:1 lipid to mRNA PCI/wt ratio

LNPs, 12 µg mRNA

PCI with 12 µg naked mRNA and 0.003 µg fimaporfin

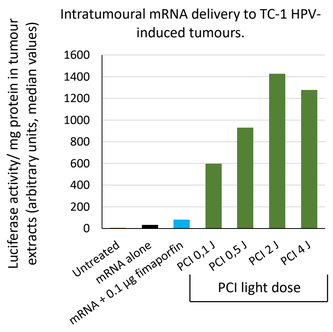
LNPs vs PCI/naked mRNA. 3 µg mRNA. Mean +/- SD.



- In the MC38 tumour model, PCI with naked mRNA performed substantially better (up to 30 times) than LNP-formulated mRNA

TC-1 mouse model for HPV induced cancer - PCI/naked mRNA and naked mRNA alone

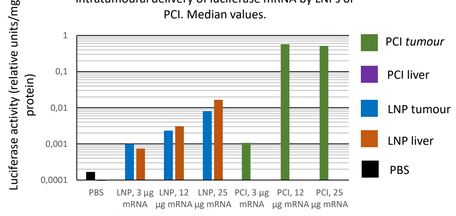
Intratumoural mRNA delivery to TC-1 HPV-induced tumours.



- In the TC-1 model, PCI increased the delivery of naked mRNA nearly 50 times as compared to naked mRNA alone

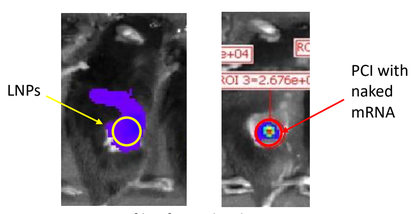
In the MC38 model, PCI with naked mRNA does not give off-target mRNA expression, and does not induce inflammatory cytokines

Luciferase expression in MC38 tumours and liver after intratumoural delivery of luciferase mRNA by LNPs or PCI. Median values.

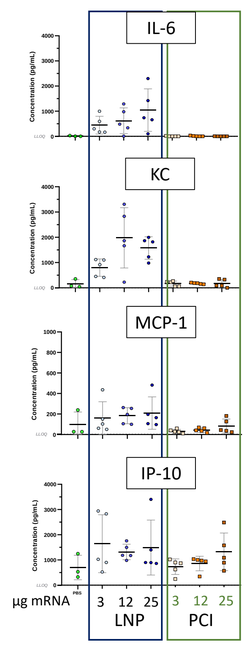


With LNPs, substantial off-target expression in liver

With PCI, no off-target expression in liver



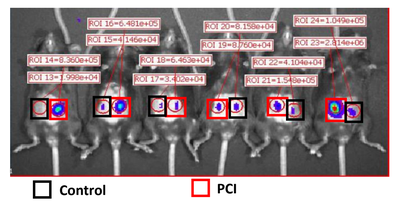
IVIS imaging of luciferase bio-luminescence



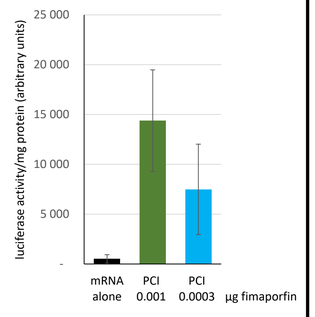
- With PCI-mediated intratumoural delivery of naked mRNA to MC38 tumours, there is no detectable leakage of functional mRNA from the tumour, and no off-target expression in the liver
- With LNPs there is substantial tumour leakage, leading to significant off-target mRNA expression in the liver

- No induction of expression of inflammatory cytokines is observed on MC38 intratumoural mRNA delivery with PCI
- LNPs induce substantial cytokine expression in the same model

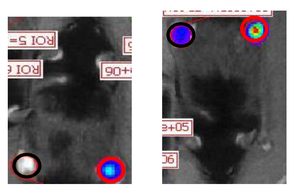
PCI strongly improves delivery of naked mRNA to skin



- PCI can enhance functional naked mRNA delivery to skin (intradermal injection) about 30 times

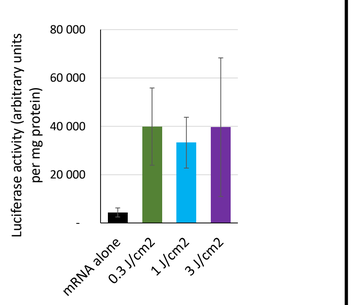


PCI strongly enhances intramuscular delivery of naked mRNA



2 µg mRNA + fimaporfin

2 µg mRNA alone



- PCI can enhance intramuscular (thigh muscle) delivery of naked mRNA about 8 times

Methods

Fimaporfin is mixed with mRNA/vehicle complexes or with naked mRNA in aqueous solution. The mixture is added to the cell medium (*in vitro*) or injected into the target tissue (*in vivo*). In *in vivo* studies control sites (usually in the same animal) are injected with the same amount of mRNA without fimaporfin, or in some experiments with LNP-formulated mRNA. 1-60 min after addition/injection the cells or injection sites (also control sites) are illuminated for 1-6 min. *In vitro,* delivery of EGFP-encoding mRNA delivery is assayed by fluorescence microscopy or flow cytometry. *In vivo,* the delivery of luciferase-encoding mRNA to target tissues is analysed by whole body bioluminescence imaging (IVIS) and by a luciferase enzymatic assay on tissue homogenates.

Conclusions

- **In a light-directed manner, PCI can enhance mRNA delivery both *in vitro* and *in vivo***
  - *In vitro*, PCI enhances mRNA delivery with many different delivery vehicles, both polymer-, lipid- and peptide based
  - *In vivo*, PCI can enhance delivery of naked mRNA to tumours, skin and skeletal muscle. Up to 50 times improvement in luciferase mRNA expression has been observed
  - PCI with naked mRNA can improve delivery to tissues/tumours where LNPs have limited activity (e.g. 30 times improvement was observed in the MC38 tumour model)
  - Fimaporfin, the active substance in PCI, is a very stable compound that can be mixed with both naked mRNA and with mRNA formulated in different delivery vehicles
  - *In vivo*, the PCI effect is induced by illumination shortly (1 - 60 min) after injection of the mRNA/fimaporfin mixture into target tissues
- **PCI and is an attractive technology for local *in vivo* mRNA delivery, especially in situations where off-target expression is a concern**
  - With PCI-mediated intratumoural naked mRNA delivery In the MC38 model there is no leakage of functional mRNA from the tumour and no off-target expression in the liver
  - In contrast, with LNPs there is substantial tumour leakage, leading to significant off-target mRNA expression in the liver