Optical delivery of ARV drugs into HIV-1 permissive cells

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What is HIV-1?
1996 - “Ongoing declines in AIDS incidence and deaths in developed nations, primarily due to widespread use of HAART”

(Roger J Pomerantz and David L Horn)
AIDS-related deaths, 1995–2011

Sub-Saharan Africa region:

23.5 mil living with HIV-1

(World Health Organisation, 2012)
People receiving HAART, 2002–2011

No. of people in millions

- North Africa and the Middle East
- Europe and Central Asia
- East, South and South-East Asia
- Latin America and the Caribbean
- Sub-Saharan Africa

(World Health Organisation, 2012)
Downfalls of HAART

- Treatment is lifelong
  - Long-term toxicities and side effects

- Emergence of drug resistance

- Poor targeting ability to latent sites
  - Lymphatic system, macrophages, CNC and lungs
Deliver anti-HIV-1 drugs using femtosecond (fs) laser pulses

- Optical delivery of therapeutic drugs has not yet been demonstrated in literature

Previous studies where method was used

- DNA plasmids – pGFP (Tirlapur & Konig, 2002)
- Viability dyes – Trypan blue (Stevenson, D et al, 2006)
- Transcription factors
- Applicability to stem cell differentiation (Mthunzi, P et al, 2010)
Objectives

- Assemble and characterise an optical translocation setup
- Optically deliver tenofovir via fs laser pulses into TZM-bl cells
- Miniaturise current drug inhibition assay protocol
  - Drug – cell exposure time
  - Cell concentration
  - Reagents used
Photo-translocation optical setup

- Koehler illumination
- XYZ translation stage
- 0.8 NA objective
- LDW objective
- CCD camera
- 1064 nm laser
- M1, M2, M3, M4
- L1, L2, TL
- Shutter

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Selective and non-invasive nature of photo-translocation

- Genetic material (DNA)
- Laser beam
- Mammalian cell
- DNA and transient hole

Microscope objective
HIV-1 inhibition assay

No infection

Tzm-bl cell

Tenofovir
Pseudovirus

2013
Emerging Researchers Symposium

our future through science
Laser-assisted drug delivery enhances HIV-1 inhibition

Table showing obtained RLU values

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<th>PC</th>
<th>Experiment</th>
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- TC – tenofovir control
- PC – positive control
- Experiment: Laser-treated cells

Graph showing RLU values of laser assisted drug delivery compared to non-treated cells
Conclusions

• Successful assembly of photo-translocation setup
• Successful photo-translocation of tenofovir into TZM-bl cells
  • Increased drug uptake
  • Reduction of drug – cell exposure time
    • 48 hours to 30 minutes

• Decreased cell concentration
  • $1 \times 10^4$ to $5 \times 10^3$

• Decreased ELISA plate well usage
  • 96 wells to 12 wells
  • Decreased reagents
Future perspectives

- Decrease diameter of sample chamber
- Further decrease in cell numbers
- Change laser beam shape

- Compare photo-translocation efficiency
- Drug delivery into multiple number of cells by incorporating SLM
- Cytotoxicity and cell viability testing
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Thank you