Shining light on cells to cure diseases

SPIE LASE
Photonics West 2017
San Francisco, CA, 29 January 2017
Nabiha Saklayen  
Marinna Madrid  
Eric Mazur
Mandal et al., Cell Stem Cell 15, 643 (2014)
white blood cells
HIV

white blood cells

Mandal et al., *Cell Stem Cell* 15, 643 (2014)
Mandal et al., Cell Stem Cell 15, 643 (2014)
from bone marrow

stem cells

gene edit

edited cells

transplant

Mandal et al., *Cell Stem Cell* 15, 643 (2014)
How can we efficiently deliver cargo to cells?
specifically, gene-editing tools to stem cells
<table>
<thead>
<tr>
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1 substrate
1. substrate

3 µm
substrate
substrate
substrate
substrate
works, but not consistently
solution: engineer substrates
solution: engineer substrates

substrate solution: engineer substrates

photoresist

Si
solution: engineer substrates

pattern

Si
solution: engineer substrates

etch

Si
solution: engineer substrates

Au deposition

Si
solution: engineer substrates

![Diagram of substrate](image)

- coverslip
- Si
solution: engineer substrates
solution: engineer substrates

pyramid substrate

Si
base: 2.4 µm
height: 1.4 µm
spacing: 3.8 µm
only exposed cells on pyramids take up dye

can porate with plasmonic substrates!
femtosecond pulses

1 substrate
2 mechanism
femtosecond pulses

1 substrate  2 mechanism
nanosecond pulses?
works too!
what mechanism?

plasmons
what mechanism?

1 substrate  
2 mechanism

plasmons → NF enhancement
what mechanism?

1 substrate

2 mechanism

plasmons → NF enhancement → poration
what mechanism?

1 substrate

2 mechanism

- plasmons
- NF enhancement (crossed out)
- poration
what mechanism?

1. substrate
2. mechanism
finite element modeling

Huber et al., submitted for publication

1 substrate  2 mechanism
Huber et al., submitted for publication
surface temperature

Huber et al., submitted for publication

1 substrate

2 mechanism
> 20 J/m²

1 substrate

2 mechanism
5.4 J/m²

1 substrate

2 mechanism
bubble formation

Chen et al., submitted for publication
bubble formation

Chen et al., submitted for publication
bubble formation

Chen et al., submitted for publication
bubble formation

Chen et al., submitted for publication
bubble formation

Chen et al., submitted for publication
bubble formation

Chen et al., submitted for publication
bubble formation and collapse

Chen et al., submitted for publication

1 substrate  2 mechanism
poration mediated by microbubbles
experimental protocol

1 substrate
2 mechanism
3 results
experimental protocol

seed HeLa cells
experimental protocol

1. substrate
2. mechanism
3. results

add calcein green
Experimental protocol

---

1. Substrate
2. Mechanism
3. Results

- Scan laser
experimental protocol

viability indicator
experimental protocol

1 substrate
2 mechanism
3 results
experimental protocol

1 substrate
2 mechanism
3 results
fluorescence microscopy

[Image: Comparison of pyramids and flat substrates under fluorescence microscopy.]

1 substrate
2 mechanism
3 results
fluorescence microscopy

1. substrate
2. mechanism
3. results
fluorescence microscopy

1. substrate
2. mechanism
3. results

- **viability**
  - calcein AM
    - 98% of cells

- **efficiency**
  - calcein green
    - 78% of cells
optimizing fluence

![Graph showing fluence vs. viability and efficiency/viability.]

1. substrate
2. mechanism
3. results

- fluence (J/m²)
- viability: 98%
- efficiency/viability (%)
optimizing fluence

1 substrate

2 mechanism

3 results

optimizing fluence

viability: 98%

viability efficiency

fluence (J/m²)

efficiency/viability (%)
optimizing fluence

1. substrate
2. mechanism
3. results

- fluence (J/m²)
- efficiency: 78%
- viability
- efficiency/viability (%)
optimizing fluence

1. substrate
2. mechanism
3. results

-optimizing fluence

<table>
<thead>
<tr>
<th>fluence (J/m²)</th>
<th>viability (%)</th>
<th>efficiency (%)</th>
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<tbody>
<tr>
<td>4.5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5.0</td>
<td>80</td>
<td>80</td>
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<tr>
<td>5.5</td>
<td>60</td>
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<td>6.0</td>
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Diagram: Efficiency/viability (%) vs. fluence (J/m²)
- Open circles: viability
- Filled circles: efficiency
optimizing fluence

![Graph showing the relationship between fluence (J/m²) and efficiency/viability (%). The graph indicates that efficiency increases with fluence, while viability decreases.]

1. substrate
2. mechanism
3. results
goal

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throughput

400 mm$^2$ in 2 s $\approx 10^6$ cells/min!

1 substrate  2 mechanism  3 results
## goal

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1. substrate
2. mechanism
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1️⃣ substrate  2️⃣ mechanism  3️⃣ results
versatility

cargo size, cell type

1 substrate  2 mechanism  3 results
cargo-size

1 substrate
2 mechanism
3 results
cargo-size

The chart illustrates the relationship between molecular weight (kDa) and efficiency/viability (%). The data points indicate a decrease in efficiency and viability as the molecular weight increases. The chart distinguishes between viability (circles) and efficiency (black dots).
cargo-size

1. substrate
2. mechanism
3. results
cell type

adherent

HeLa

breast

muscle
cell type

adherent

- HeLa
- breast
- muscle
cell type

adherent

HeLa

breast

muscle

substrate  mechanism  results
cell type

adherent

- HeLa
- breast
- muscle
cell type

adherent

- HeLa
- breast
- muscle

suspension

- bone marrow
- immune
- stem
cell type

adherent

HeLa

breast

muscle

suspension

bone marrow

immune

stem

substrate  mechanism  results
### Goal

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can deliver CRISPR-Cas9-sized molecules to suspension cells
from bone marrow

stem cells

gene edit

edited cells

transplant
T cells from blood stream
from blood stream

T cells

gene edit

edited cells

from blood stream
from blood stream
T cells
gene edit
edited cells
from blood stream
reinject
plasmonic substrates are reshaping intracellular delivery