Genetic transformation of cells using physical methods

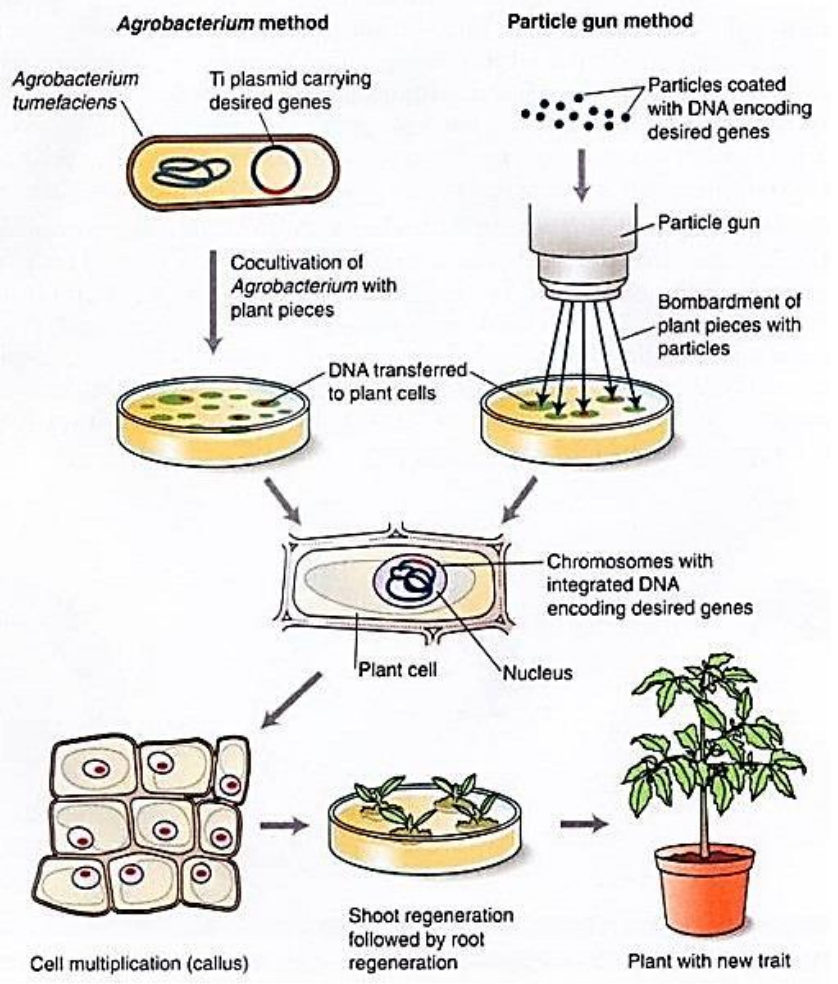
Ana Leonor Rivera

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Abstract

We present a general panorama of the physical methods used for genetic transformation of cells, making a comparison of their advantages and drawbacks.



Ana Leonor Rivera

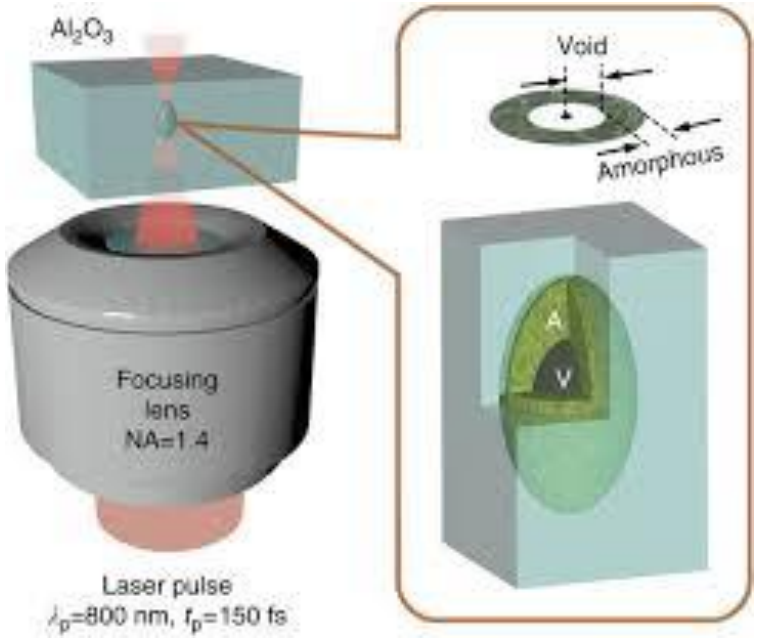
Outline

- **Introduction**: Why to perform physical methods to genetically transform cells?
- **Physical transformations**
- **Traditional popular methods**:
  - Electroporation
  - Biolistics



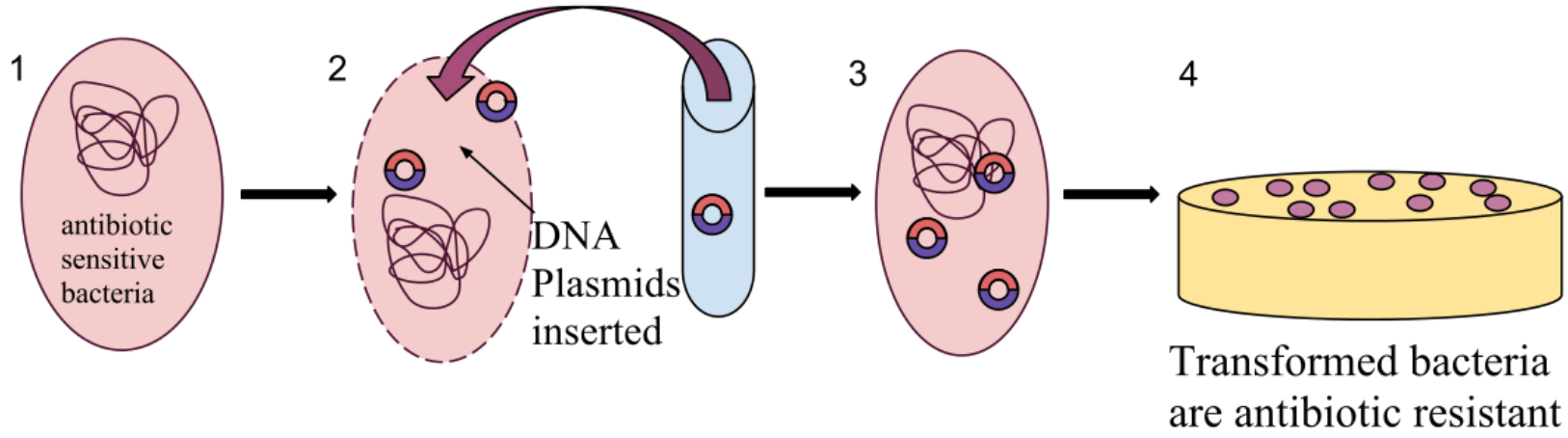
Outline

- **Less common methodologies**:
  - Agitation with glass beads
  - Vacuum infiltration
  - Silicon carbide whisker
  - Laser manipulation
- **New techniques**:
  - Shock waves
- **Conclusion**



Introduction:

Why to perform physical methods to genetically transform cells?



Why to perform physical methods to genetically transform cells?

- To insert genes from plants, fungi, viruses, bacteria and even animals into cells,
- to insert enzymes, metabolites, lipids and pharmaceutical compounds,
- to identify sequences that confer resistance to antibiotics (selective markers),
- to elaborate DNA vaccines,

PJ Canatella et al. (2001) *Gene Ther* **8**:1464.
L Frelin et al (2010) *Drug News Perpect* **23**:647.

Why to perform physical methods to genetically transform cells?

- to produce enzymes that generate a specific property not observed in the wild type strain (reporter genes),
- to characterize genes involved in a metabolic route,
- for cancer treatment,

JM Lee et al. (2013) *J Mammary Gland Biol Neoplasia* **18**:233.
D. Miklavi et al. (2012) *Med Biol Eng Comp* **50**:1213.

Why to perform physical methods to genetically transform cells?

- To transform from one cell type to another (reprograming cells).

Example: transformation of human scar-tissue into heart muscle cells. Fibroblasts could be reprogrammed into beating heart cells by injecting 3 genes GMT mix plus genes ESRRG & MESP1 (for complete transform MYOCD & ZFPM2).

L Qian et al. (2012) *Nature* **485**:593.
JD Fu et al. (2013) *Stem Cell Rep* 22.

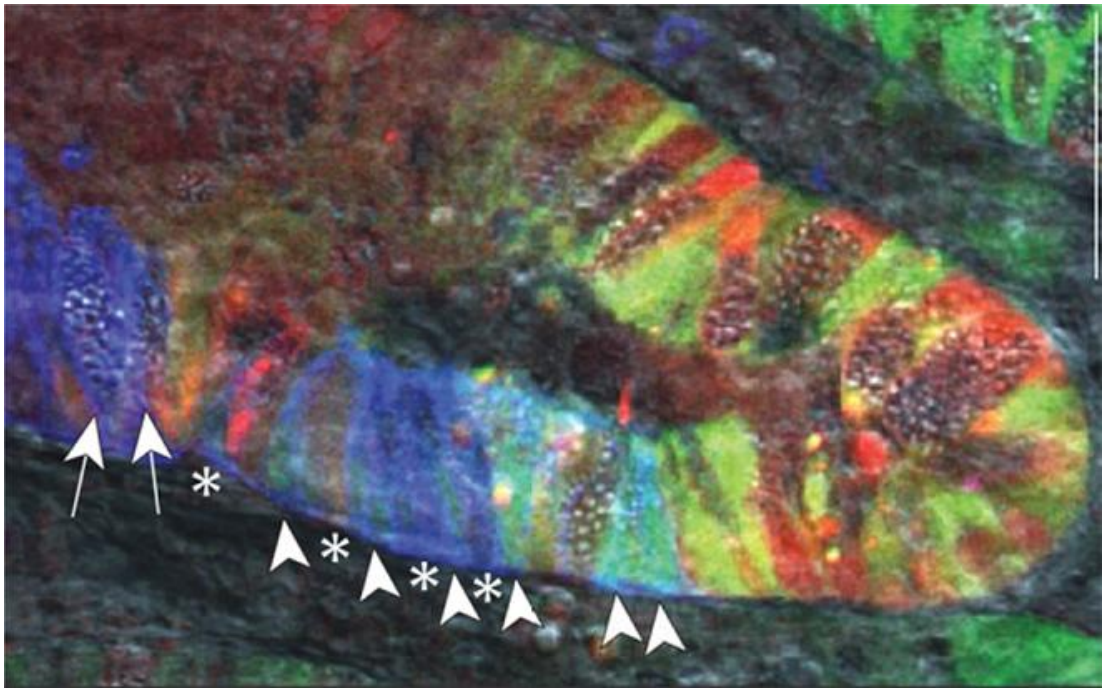
Requirements for genetically transformation of cells

- Production of recombinant DNA fragments
- transferring of the DNA into the cell by membrane permeabilization,
- integration of the DNA into a chromosome and its maintenance and replication.

For genetically transformation of cells it is necessary:

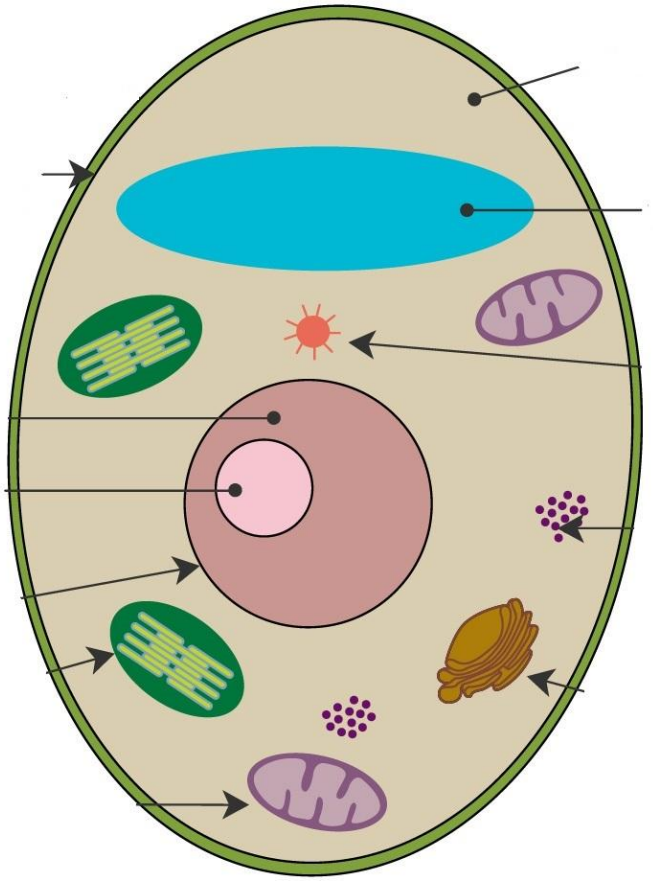
- in vitro culture for multiplication of clones,
- to select suitable promoters for a specific gene,
- over-expressing activator genes,
- removing epigenetic silencing,
- introducing heterologous genes,
- improving bioinformatics programs of random mutagenesis.

Physical transformation



Genetic transformation

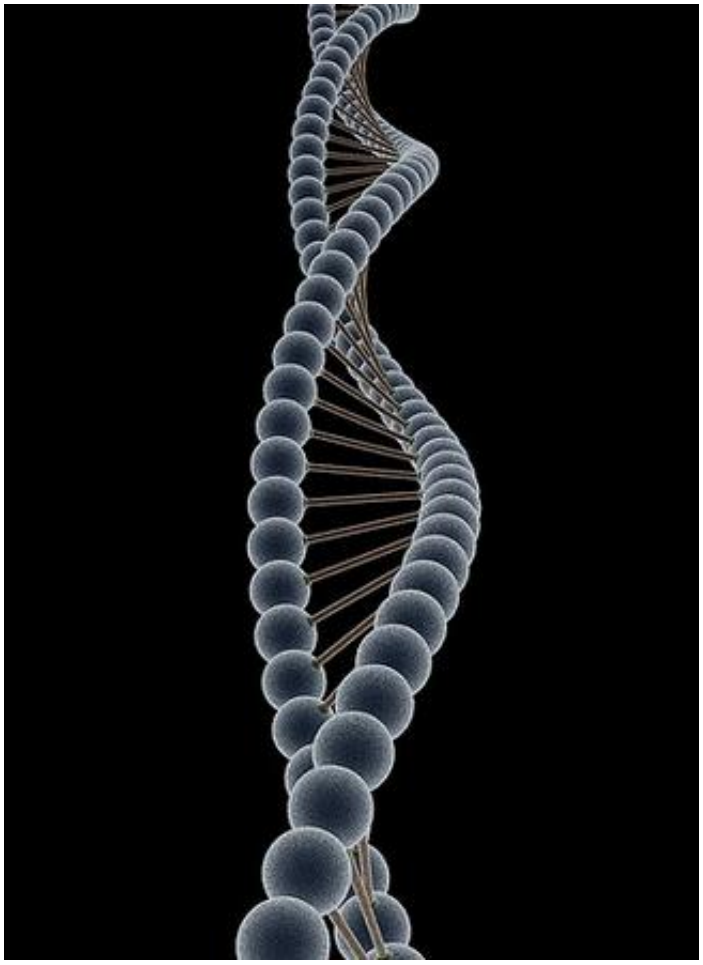
It requires penetration of the transgene through the cell membrane (hydrophobic layer ≈10nm).



M Meselson & R Yuan (1968) *Nature* **217**:1110.
AL Rivera et al. (2012) *Phys Life Rev* **9**:308.
AL Rivera et al. (2014) *Phys Life Rev* **11**:184.

Genetic transformation obstacles

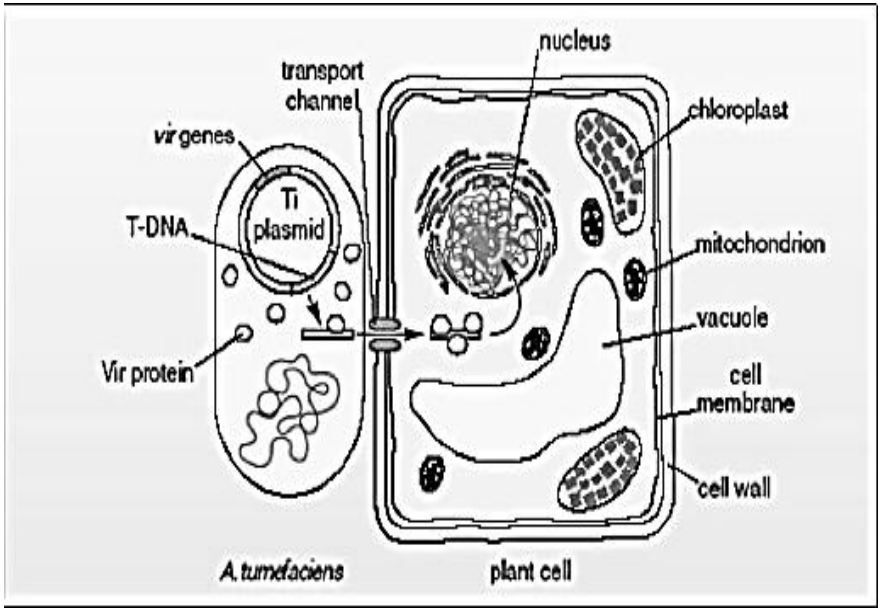
DNA is a highly charged macromolecule, difficult to manipulate, that cannot diffuse through the cell membrane.



Electron microscope false colored DNA image from https://www.flickr.com/photos/13338351@N07/2244645930.

Genetic transformation techniques

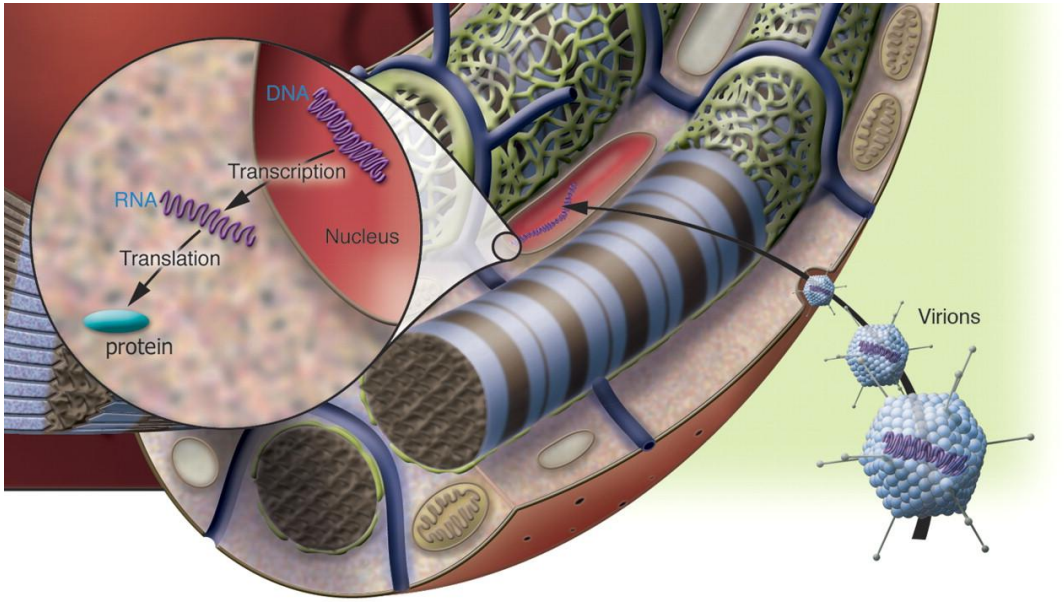
- Biological
  - *Agrobacterium tumefaciens*-mediated transformation
  - protoplast transformation using cell wall degrading enzymes



SB Gelvin (2009) *Plant Physiol* **150**:1665.

Genetic transformation techniques

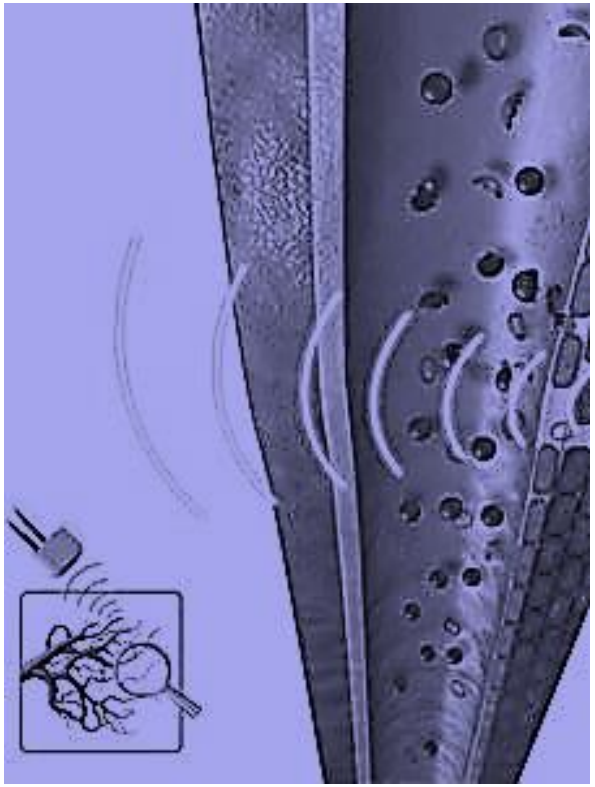
- Chemical
  - calcium phosphate coprecipitation
  - lipofection



EH Chowdhury et al. (2004) *Gene* **341**:77.
Y Kawata et al. (2003) *Biosci Biotechnol Biochem* **67**:1179.

Genetic transformation techniques

- Physical
  - Electroporation
  - Biolistics
  - Agitation with glass beads
  - Vacuum infiltration
  - Silicon carbide whisker
  - Laser manipulation
  - Ultrasound
  - Shock waves



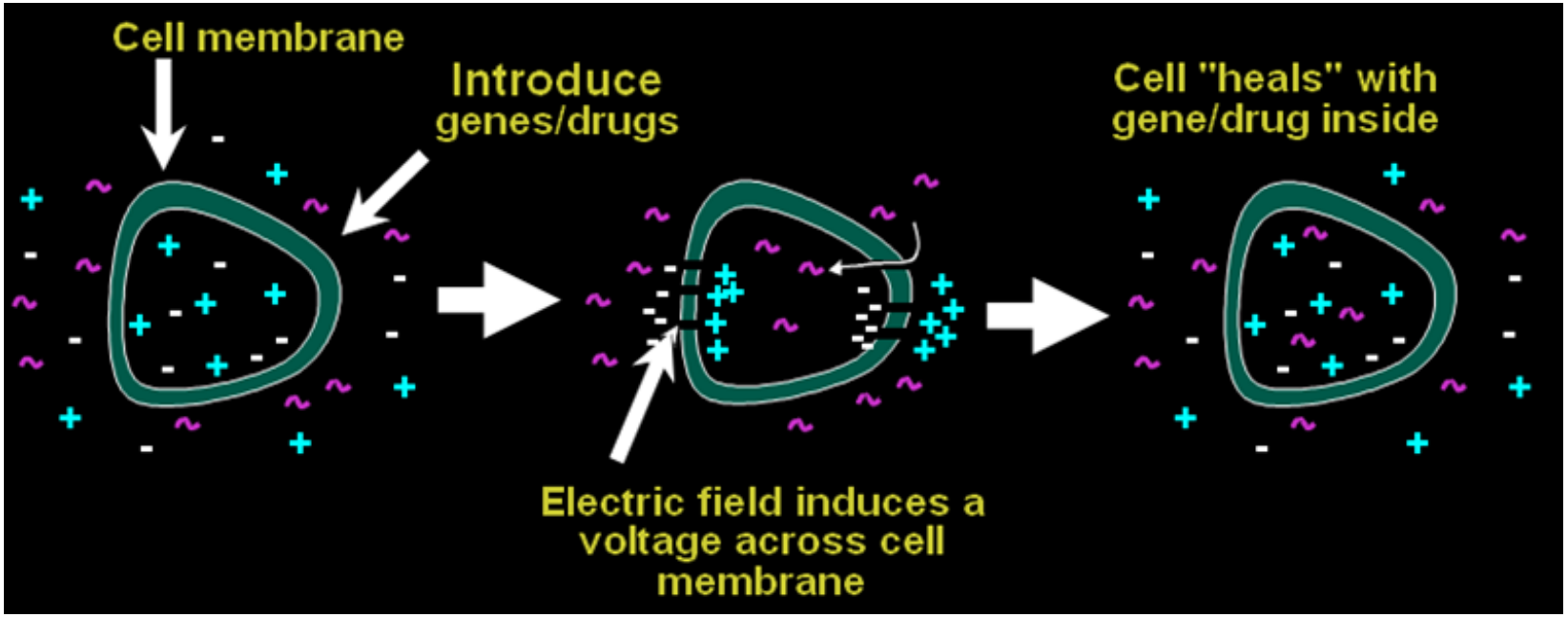
AL Rivera et al. (2014) *J Gen Syndr Gene Ther* **5**:237.

Traditional popular methods:

- Electroporation
- Biolistics

Electroporation

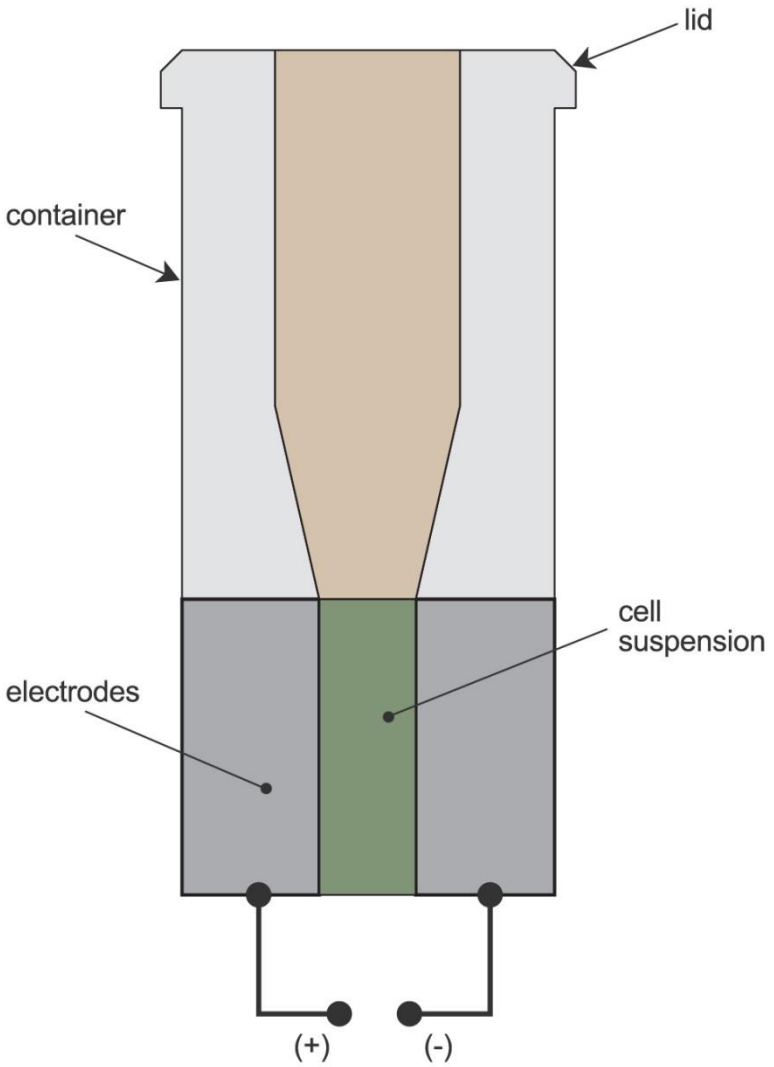
DNA is inserted through pores due to permeabilization of the cell membrane induced by a strong electrical pulses.



H Potter (1984) *Proc Natl Acad Sci USA* **81**:7161.

Electroporation parameters

- Pulse length, energy and duration of the electrical field,
- extent and duration of membrane permeation,
- mode and duration of molecular flow,
- DNA concentration,
- tolerance of cells to membrane permeation.



JM Escoffre et al. (2009) *Mol Biotechnol* **41**: 286.

Electroporation

Advantages

- Simple,
- fast,
- low cost.

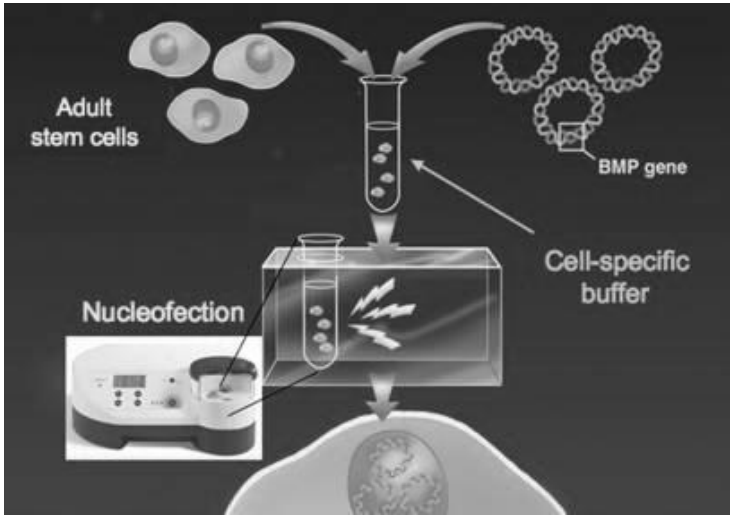
Drawbacks

- Low efficiency,
- requires laborious protocols,
- transforms mainly protoplasts.

AL Rivera et al. (2014) *J Gen Syndr Gene Ther* **5**:237.

Nucleofection

- Successful in cancer studies and tissue engineering.
- Voltage, frequency, pulse duration are not disclosed to user.
- Allows primary cells, cell lines and stem cells transfection.



HI Trompeter et al. (2003) *J. Immunol Meth* **274**:245.
M Freeley & A Long (2013) *Biochem J* **455**:133.

Biolistics
(particle bombardment or gene gun)

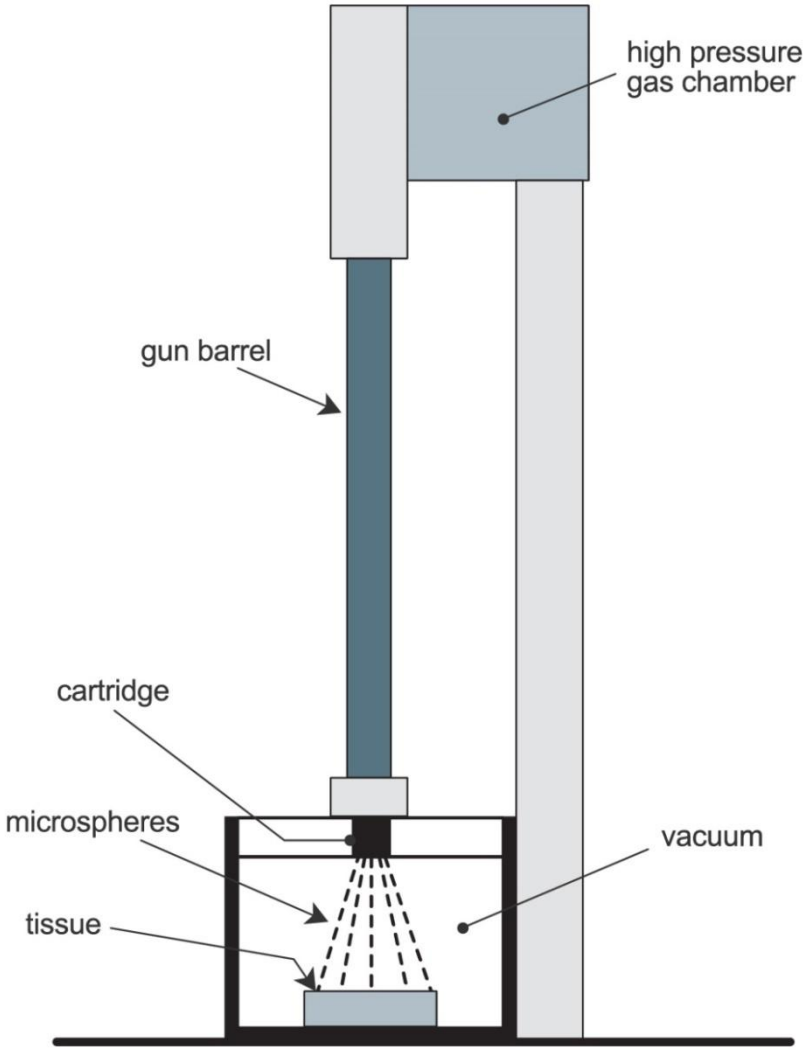
High density carrier particles covered with genes are accelerated through the cells leaving the DNA inside by an adsorption mechanism.



JC Sanford (1987) *Sci Technol* **5**:27.

Biolistics parameters

- Kinetic energy of the bombarding particles,
- temperature,
- amount of cells,
- cell‘s ability to regenerate,
- susceptibility of the tissue,
- number of DNA-coated particles,
- amount of DNA that covers each particle.



JC Sanford (2000) *In Vitro Cell Dev* **36**:303.

Biolistics

Advantages

- Simple,
- no need to treat the cell wall,
- allows transformation of different cells,
- independent of the physiological properties of the cell,
- allows the use of multiple transgenes.

Drawbacks

- Expensive,
- Low efficiency,
- Transformation parameters must be optimized to each biological target.
- Risk of multiple copies of the introduced genes that cause gene silencing or altered gene expression,
- DNA and cells can be damage

AL Rivera et al. (2014) *J Gen Syndr Gene Ther* **5**:237.

Less common methodologies:

- Agitation with glass beads
- Vacuum infiltration
- Silicon carbide whisker
- Laser manipulation

Agitation with glass beads

Rapid agitation with glass beads allows the penetration of the plasmid DNA.



MC Costanzo & TD Fox (1988) *Genetics* **120**:667.

Agitation with glass beads parameters

- DNA concentration,
- sensitivity of the cells to membrane penetration,
- amount of cells and
- cellule's ability to regenerate.



P Rattanachaikunsopon et al. (2009) *Braz J Microbiol* **40**:923.

Agitation with glass beads

Advantages

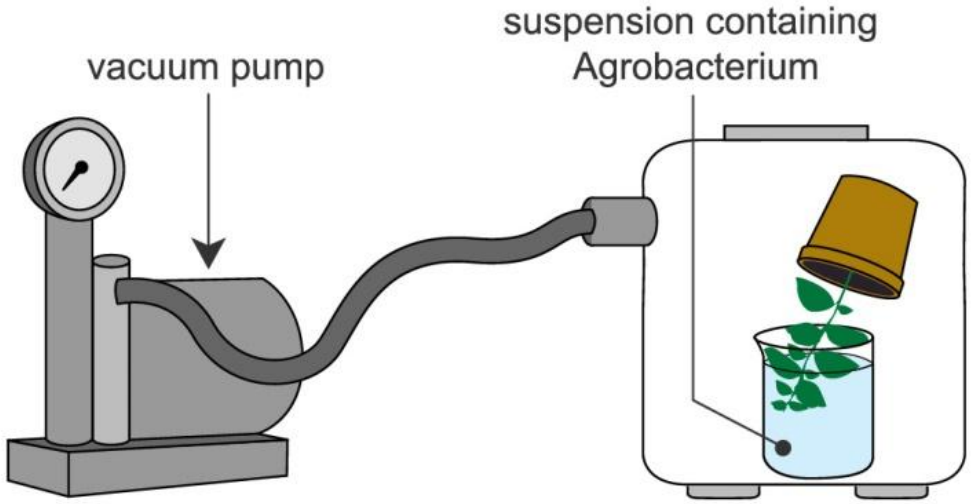
- Simple,
- cheap,
- unnecessary chemical treatments, enzymatic cocktails or sophisticated devices,
- fast.

Drawbacks

- Low efficiency,
- DNA can be damage.

Agrobacterium infiltration mediated by vacuum

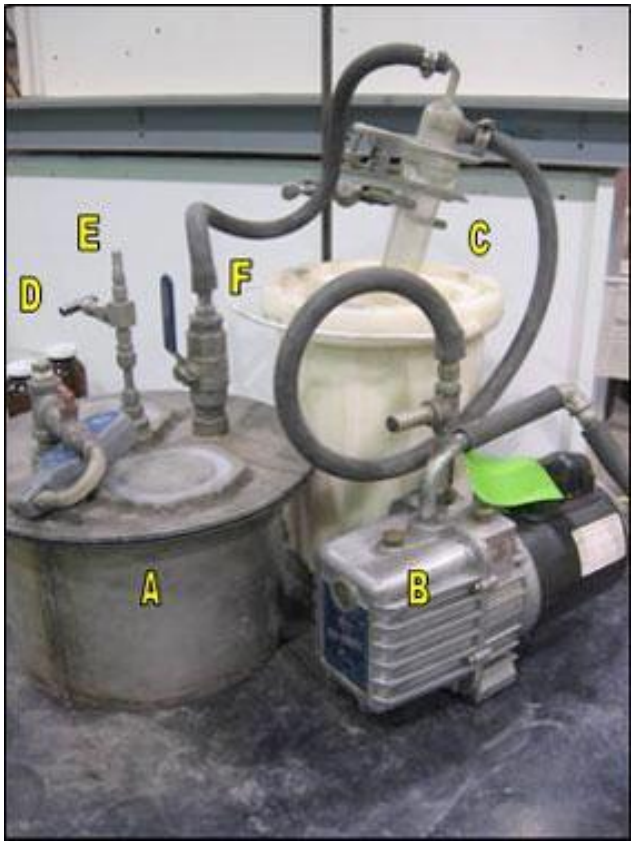
Vacuum generates a negative atmospheric pressure that increases the air spaces between cell's membrane allowing *Agrobaterium* penetration.



P Bundock et al. (1995) *EMBO J* **14**:3206.

Vacuum infiltration parameters

- Duration and type of the air pressure,
- temperature,
- pH,
- time or induction of genes.



CB Michielse et al. (2005) *Curr Genet* **48**:1.

Vacuum infiltration

Advantages

- Simple,
- Fast,
- Low somaclonal variation,
- Many independent cells can be transformed.

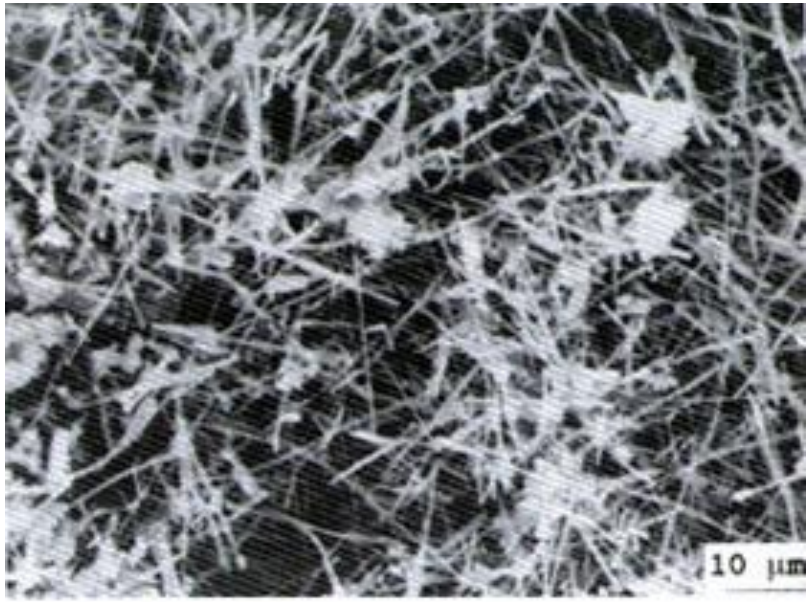
Drawbacks

- Some strains of *Agrobacterium* are unable to infect certain cell types,
- risk of multiple copies of the introduced genes that cause gene silencing or altered gene expression.

AL Rivera et al. (2014) *J Gen Syndr Gene Ther* **5**:237.

Silicon carbide whisker

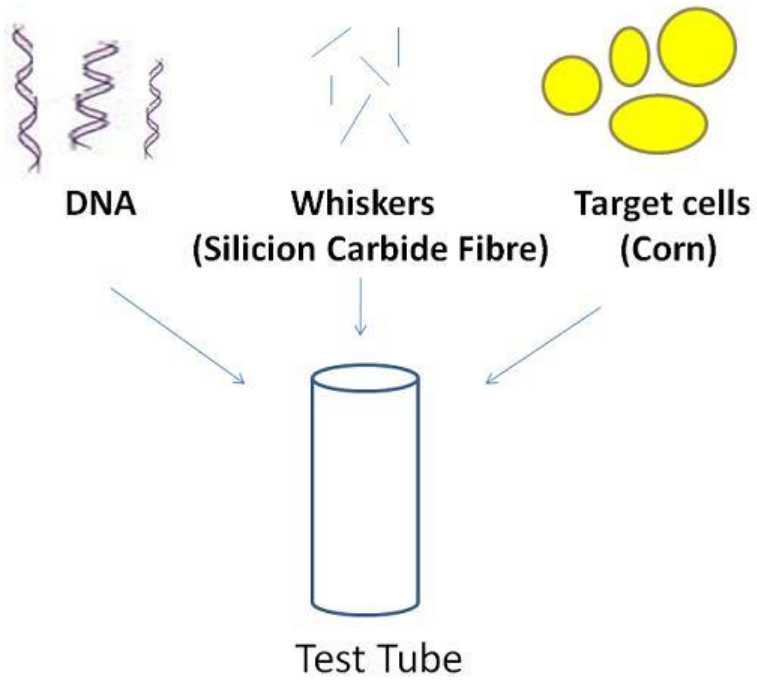
Silicon carbide fibres are capable of puncturing cells without killing them, so they are mixed in a vortex with a suspension of tissue and DNA allowing its introduction by abrasion.



HF Kaeppler et al. (1990) *Plant Cell Rep* **9**:415.

Silicon carbide whisker parameters

- Fibre size,
- vortex parameters (type, duration and speed of agitation),
- vessel shape,
- thickness of the cell wall,
- cell's ability to regenerate.



M Rakoczy-Trojanowska (2002) *Cell Mol Biol Lett* **7**:849.

Silicon carbide whisker

Advantages

- Simple,
- cheap,
- can be used in different cell types,
- fast.

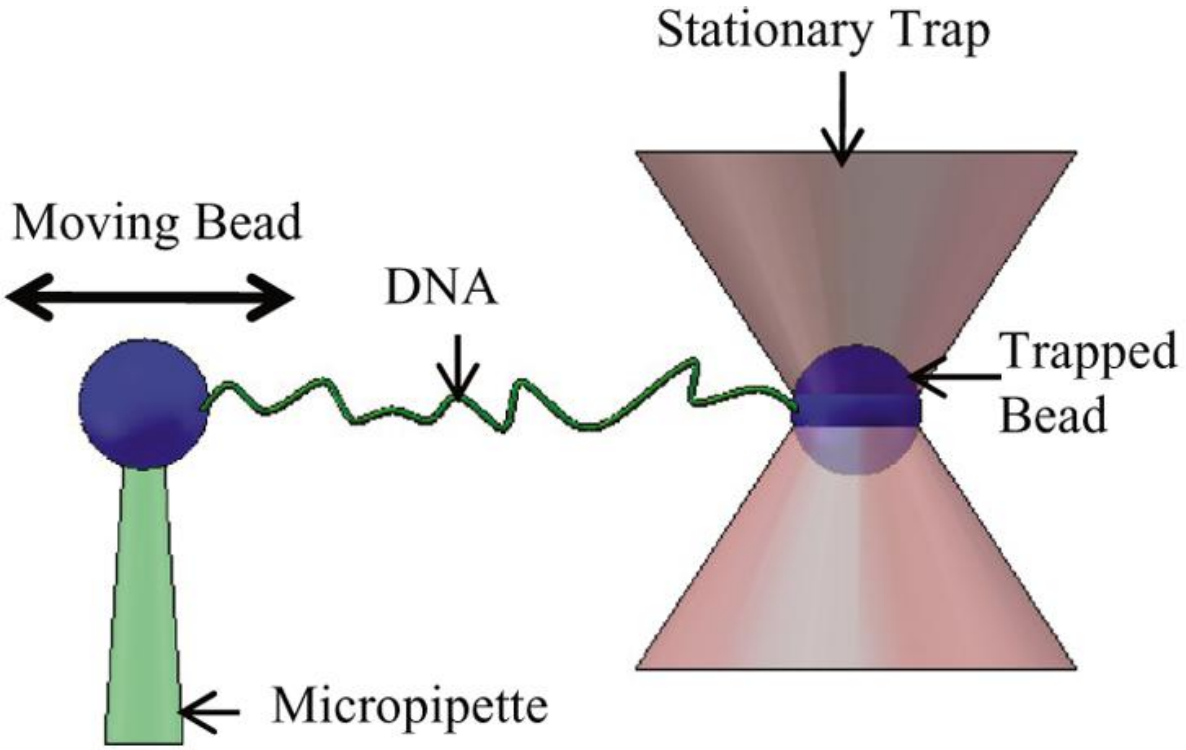
Drawbacks

- Very low efficiency,
- cells can be damage affecting regeneration capacities.
- It could be hazardous to technicians due to fibers' inhalation.

AL Rivera et al. (2014) *J Gen Syndr Gene Ther* **5**:237.

Laser manipulation: microinjection

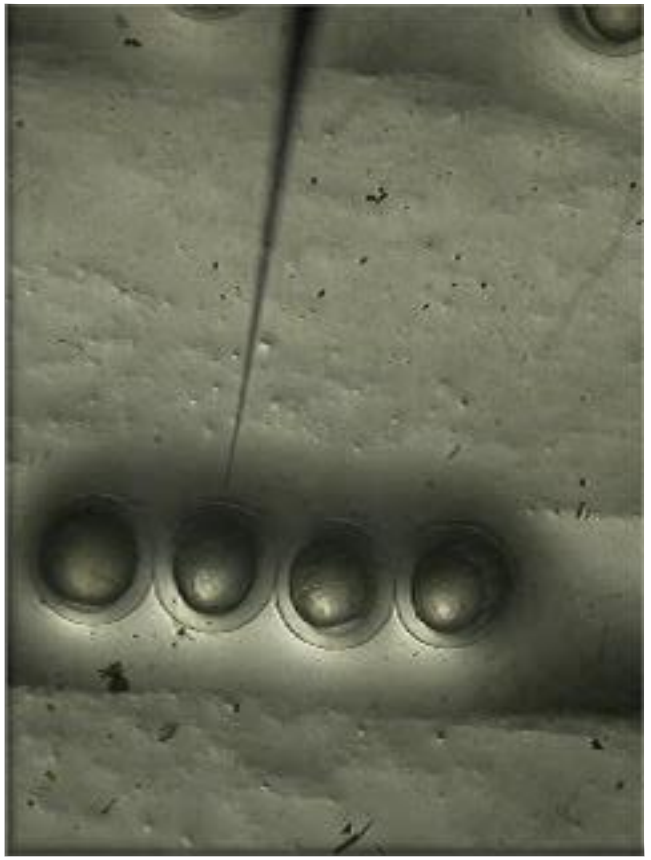
A laser microbeam punctures self-healing holes into the cell wall allowing DNA penetration.



MW Berns et al. (1981) *Science* **213**:505.

Microinjection parameters

- Laser characteristics of the optical tweezer coupled to the appropriate microscope.



KO Greulich (2000) *J Microsc* **198**:182.

Microinjection

Advantages

- Allows precise and gentle treatment of cells, subcellular structures, and even individual DNA molecules.

Drawbacks

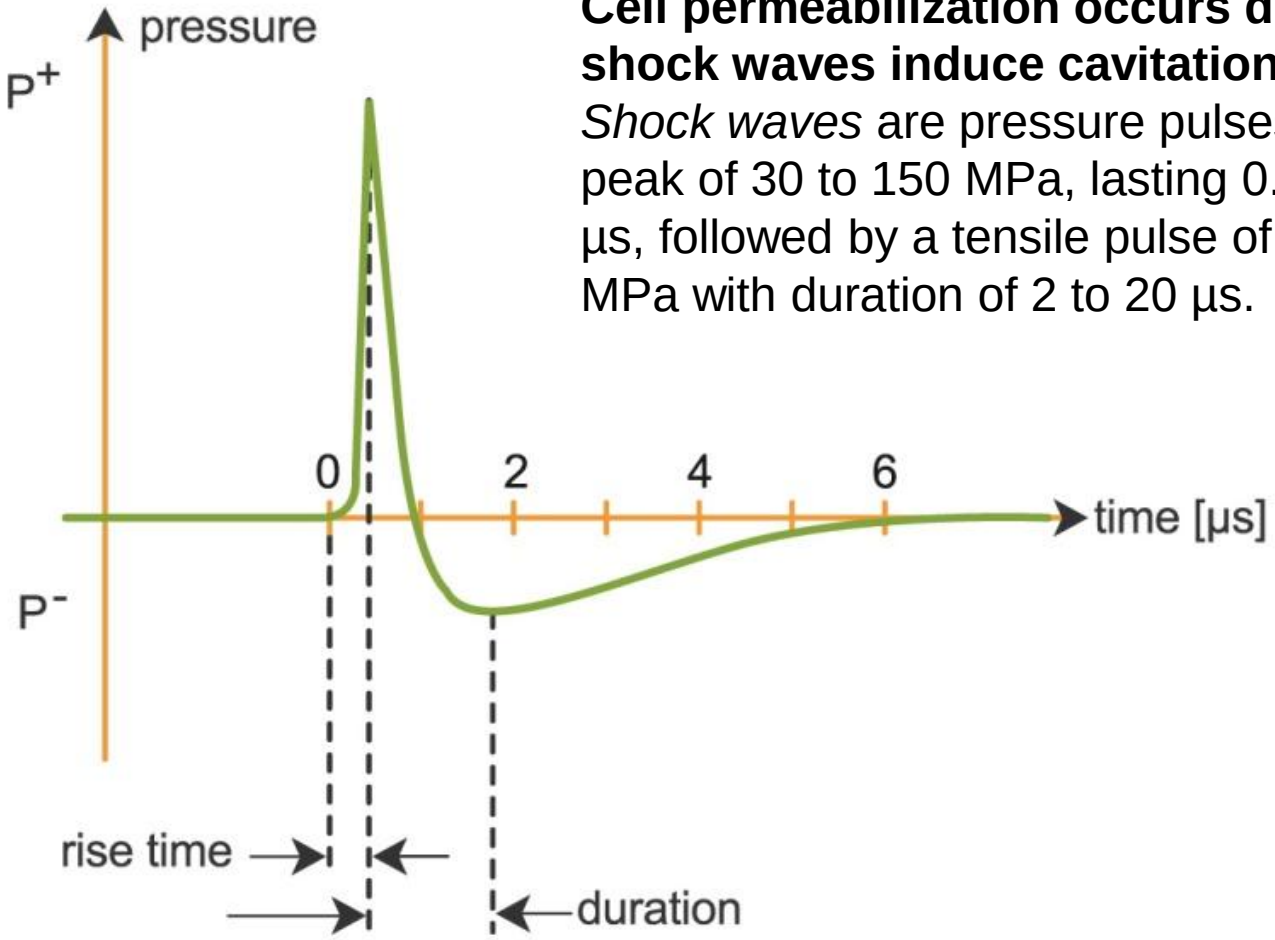
- High cost (expensive equipment required),
- Laborious protocols.

AL Rivera et al. (2014) *J Gen Syndr Gene Ther* **5**:237.

New techniques:

- Shock waves

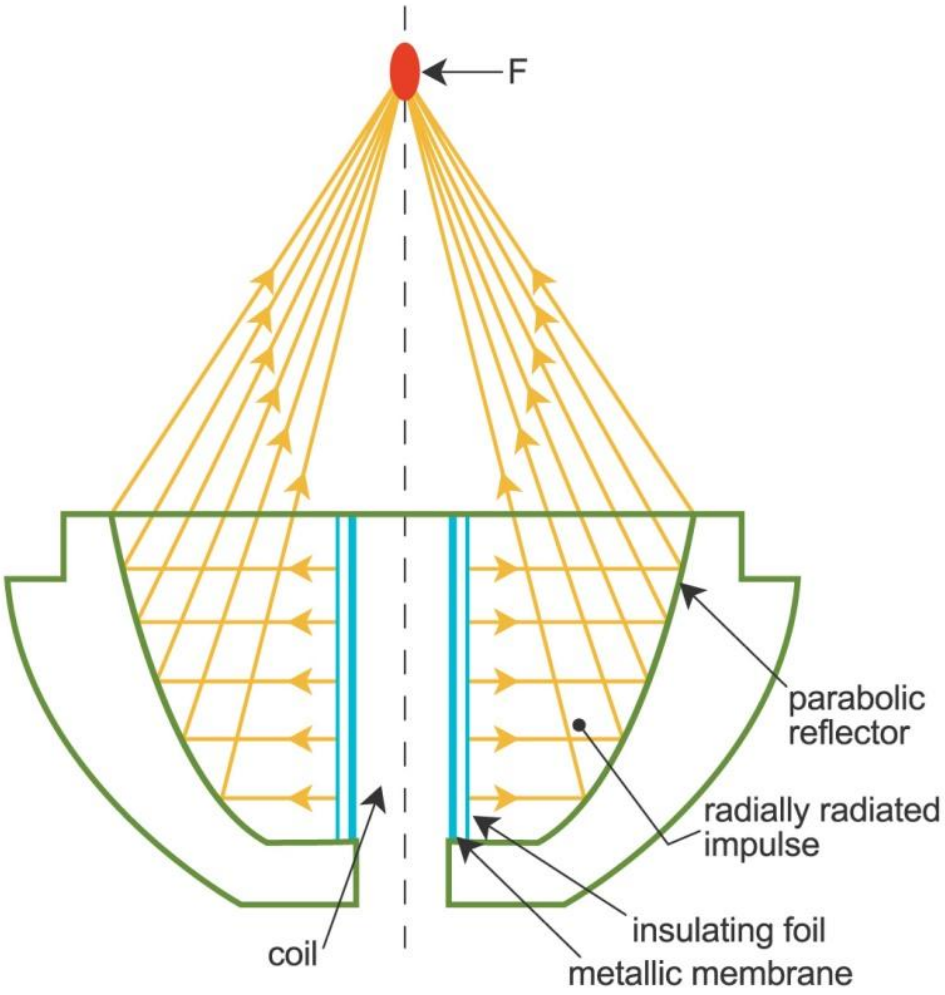
Shock wave mediated transform

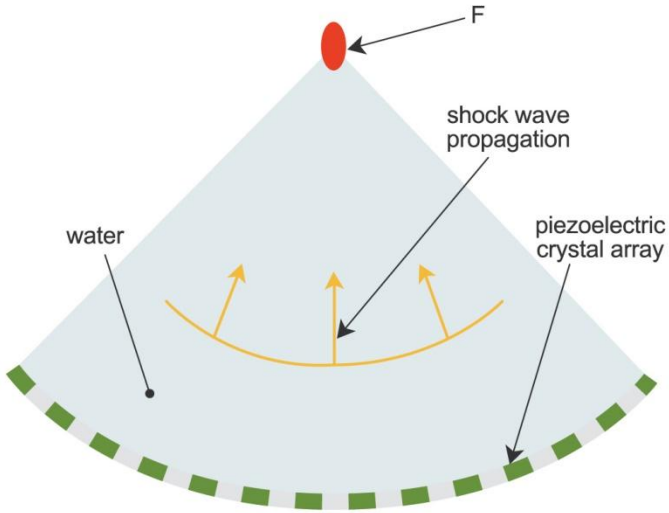


Cell permeabilization occurs due to shock waves induce cavitation.
Shock waves are pressure pulses with a peak of 30 to 150 MPa, lasting 0.5 and 3 µs, followed by a tensile pulse of up to -20 MPa with duration of 2 to 20 µs.

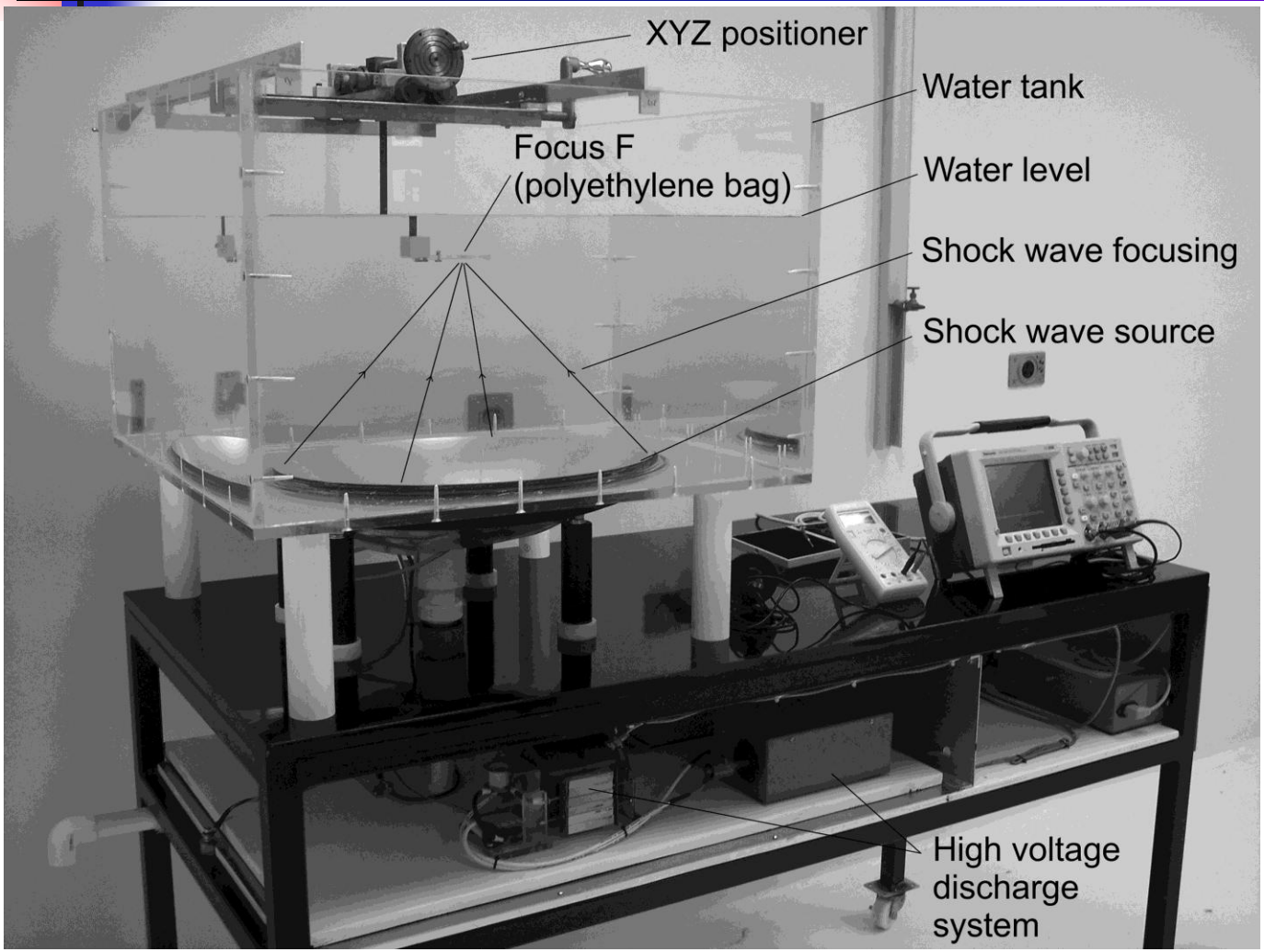
AM Loske et al. (2011) *Ultrasound Med Biol* **37**:502.

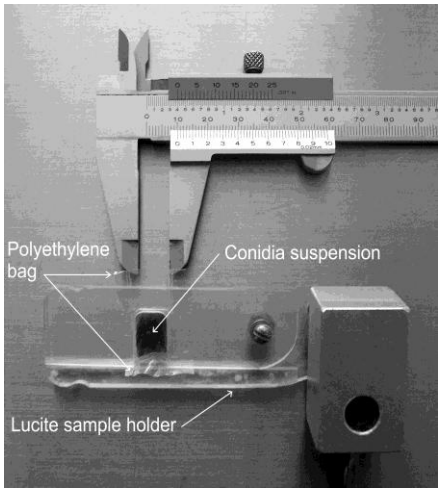
Shock wave mediated transform





Shock wave mediated transform

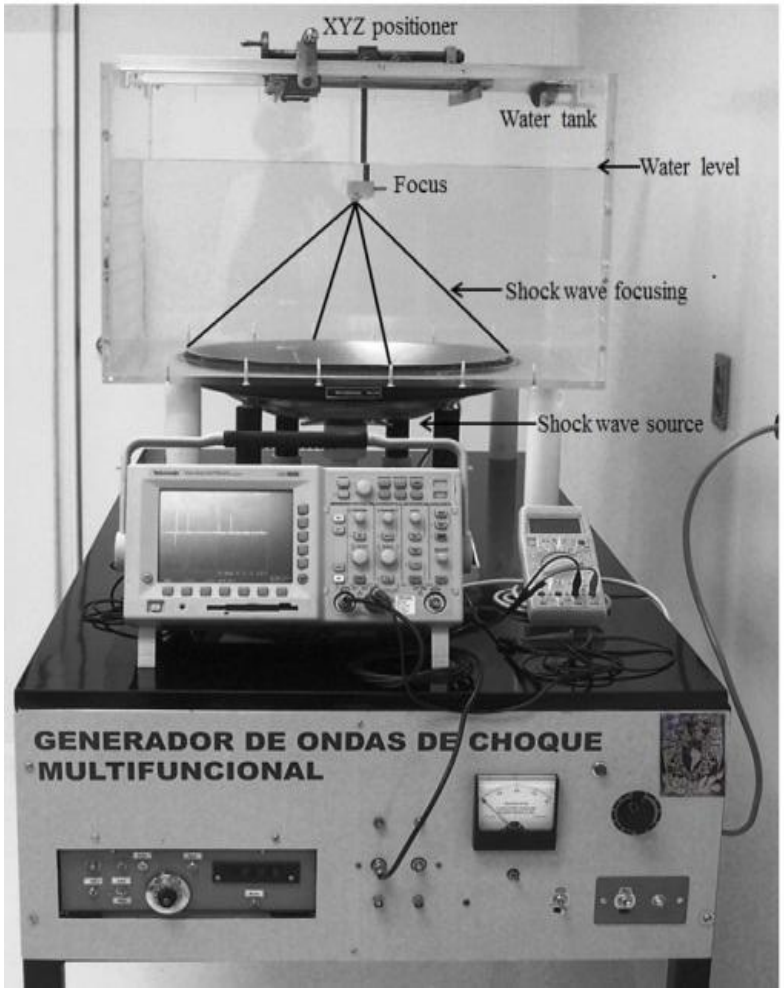




AL Rivera et al. (2012) *Phys Life Rev* **9**:308.
AL Rivera et al. (2014) *Phys Life Rev* **11**:184.

Shock wave mediated transform parameters

- Frequency
- Energy
- Voltage
- Shock wave profile
- Number of shock waves



AL Rivera et al. (2014) *Phys Life Rev* **11**:184.

Shock wave mediated transform

Advantages

- Fast,
- easy to perform,
- reproducible with high efficiency,
- no need of enzymatic cocktails,
- can be used to transform several cell types.

Drawbacks

- Shock wave generators for this purpose are not on the market yet and experimental devices are relatively expensive.

AL Rivera et al. (2014) *J Gen Syndr Gene Ther* **5**:237.

Conclusion

Efficient transformation techniques to control gene expression are needed to increase production levels and to ensure product quality.

Shock-wave mediated transform is a promising technique not yet employed for human cells.

Group team

- Denis Magaña
- Francisco Fernández
- Miguel Gómez-Lim
- Achim M. Loske



T H A N K Y O U

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- Rivera et al, “Genetic transformation of cells using physical methods” *Journal of Genetic Syndromes & Gene Therapy* **5**:237 (2014)
- Rivera et al, “Physical methods for genetic transformation of fungi” *Physics of Life Reviews* **11** # 2:184 (2014).
- Rivera et al, “Physical methods for genetic plant transformation” *Physics of Life Reviews* **9** # 3:308 (2012).