Formation of Microcraters for Gene Transfer on Plant Cell Walls by Plasma Immersion Ion Implantation

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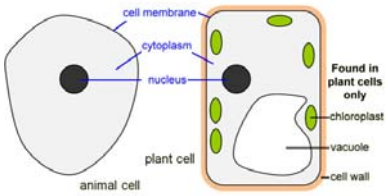
Background and Objectives

Background

- Plant cells differ from animal cells by their thick and rigid cell wall
- The cell wall prevents standard approaches of DNA transfer for genetic transformation
  - ⇨ Stripping of the cell wall (protoplast formation) for DNA transfer works only with some plants
- Perforation of the cell wall by ion beam bombardment and subsequent cell transformation have been reported[1].
  - ⇨ The ion implantation leads to characteristic **"microcraters"** in the cell wall
  - ⇨ Microcraters form pathways for penetration of DNA macromolecules.



Source: BBC.co.uk

Aim of the Study

Formation of similar "microcraters" in plant cells by means of Plasma Immersion Ion Implantation (PIII)

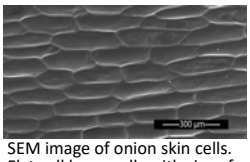
Advantage
- Less demanding technology than ion beam implantation

Problems to be solved
- low conductivity of plant cells
- Low ion penetration compared to cell wall thickness (~100 nm)

[1] S. Sangyuenyongpipat *et al.*, Nucl. Instr. Meth. Phys. Res. B **227,** 289 (2005)

Method

Target cells for ion implantation:
Onion (*Allium cepa* L.) skin cells:
0.5 cm² pieces of flat coherent cell monolayer



SEM image of onion skin cells. Flat cell layer, cells with size of some hundred microns

PIII conditions

Plasma:
13.56 MHz rf discharge argon plasma
Typical power: 400 W

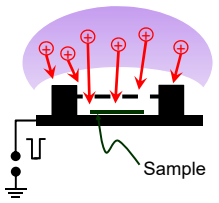
Pulses:
20 kV, 10µs wide, repetition 100 s^{-1}

Ion doses:
(1 × 10^{14}, 1 × $10^{15}$and 5 × 10^{15}) cm^{-2}

1 m cylindrical PIII chamber for the ion implantation

Implantation in non-conductive onion skin

1. Direct implantation
2. ~8 nm gold sputter deposition of the surface
3. "mesh assisted" implantation through a conductive cage at low distance[1]

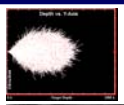


Sketch of "cage implantation" set up

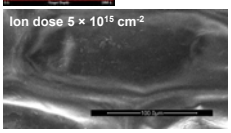
[1] R. K. Y. Fu *et al.*, J. Appl. Phys. 95, 3319 (2004)

Results

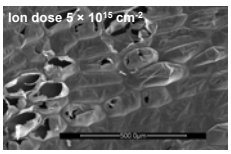
Theoretical implantation depth
SRIM simulation Ar^+ implantation into cellulose: **60 nm**



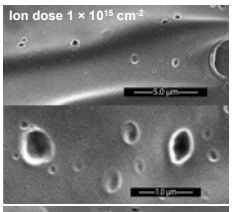
Direct ion bombardment
No change of cell morphology in SEM
⇨ Charging of surface prevents ion implantation



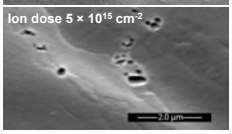
Ion bombarding on gold coated onion skin
Severe erosion and damage of the cells
⇨ Destroyed cells appear blown up



"Mesh assisted" implantation
Localized formation of "microcraters" of abt. 0.1 – 1 µm diameter at ion doses of (1 – 5) × 10^{15} cm^{-2}



Appearance of blisters at the 5 × 10^{15} cm^{-2} implanted cell layer as origin of the microcraters



Interpretation

- Observed damage (craters) is deeper than penetration range of the ions
- Ar^+ implantation dose 1 × 10^{15} cm^{-2} into a layer of 60 nm presents 6 times Loschmidt's constant N_L, causing a pressure of 6 atm in the cell wall at 0°C (out-diffusion ignored). Additional pressure arises from evaporated cell wall material.
- Blown-up appearance of gold coated cells and blister-like changes in the cell wall after 5 × 10^{15} cm^{-2} "mesh assisted" implantation support the concept of gas accumulation in the cell wall

⇨ **Microcraters are interpreted as explosive release of the accumulated gas in the cell wall**

Conclusions

- Previously observed "microcraters" in plant cell walls obtained by ion beam implantation, required for ion beam assisted gene transfer, could be reproduced by plasma based ion implantation
- Concept of microcrater formation by "explosive" pressure release is enforced
- "Mesh assisted" implantation allows treatment of low conductive biological material

FORMATION OF MICROCRATERS FOR GENE TRANSFER ON PLANT CELL WALLS BY PLASMA IMMERSION ION IMPLANTATION

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Possible techniques for the transfer of genetic material into animal cells range from viral transfection to chemical treatment and electroporation. The repertoire is more limited for bacteria or plant cells with thick and rigid cell walls, and ion beam bombardment has been used for permeabilization of the cell wall in this case. The treatment is accompanied by the formation of "microcraters", enabling subsequent DNA transfer. Most likely these microcraters are formed by explosion of bubbles of implanted gas ions and dissociated organic material, and provide a pathway into the cell interior.

We have demonstrated the use of plasma immersion ion implantation as a means for carrying out ion bombardment for this purpose, as an alternative to the more-demanding beam line implantation technique. The low conductivity of the biological target material, the wide energy distribution of the impinging ions, and sputtering of the soft organic cell wall material were the demanding points of the task.

Onion skin was used for implantation because these cells are thick walled, flat, and easy to prepare as an intact monolayer. The cell sample was covered by a wire mesh at a distance of 2-3 mm to overcome charging effects of the insulating surface. Ar ion bombardment was performed using 20 kV piii pulses with a dose of about 1×10^{15} ions/cm^2. Scanning electron microscopy subsequent to the piii treatment revealed the formation of a low density of microcraters on the otherwise intact cell walls. Implantation at higher dose indicated blister formation.

We conclude that piii can substitute for beam line implantation for cell permeabilization by energetic ion bombardment.

Poster presentation preferred