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Cold gas plasma in medicine and biology

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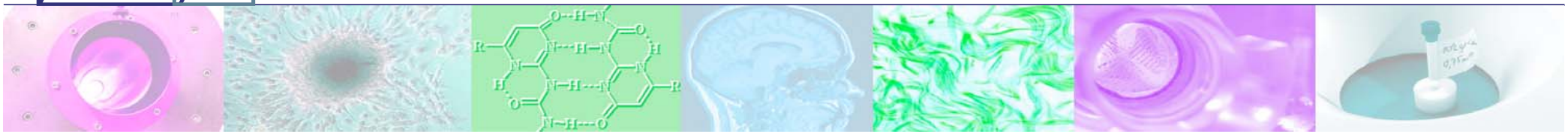

Cold gas plasma in medicine and biology

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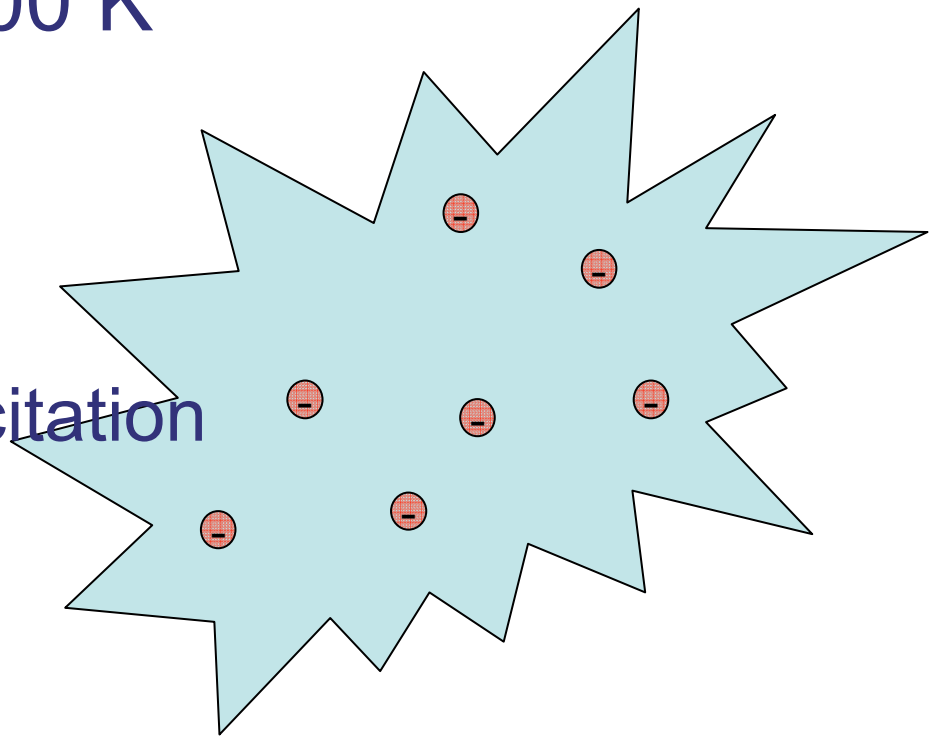
With many thanks to the team: Ingrid Kieft, Raymond Sladek, Tom Baede, Robin v. Gastel, Evert Ridderhof, Maarten Steinbuch, Dick Slaaf

Eindhoven University of Technology

www.bmt.tue.nl/plasma

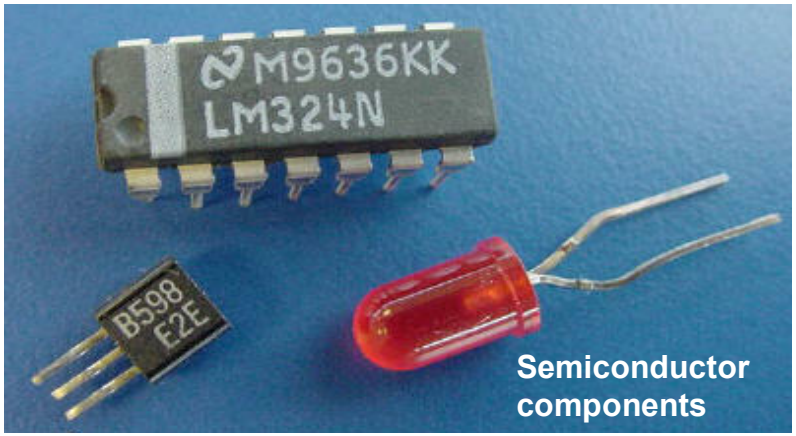
Cold plasma

- Ionised gas, non-equilibrium
- Electrons > 1 eV, gas < 400 K
- When does it happen?
  - Low power
  - Low pressure
  - High-frequency electric excitation
  - Small plasma size
  - Short power pulses
  - Convective/other cooling

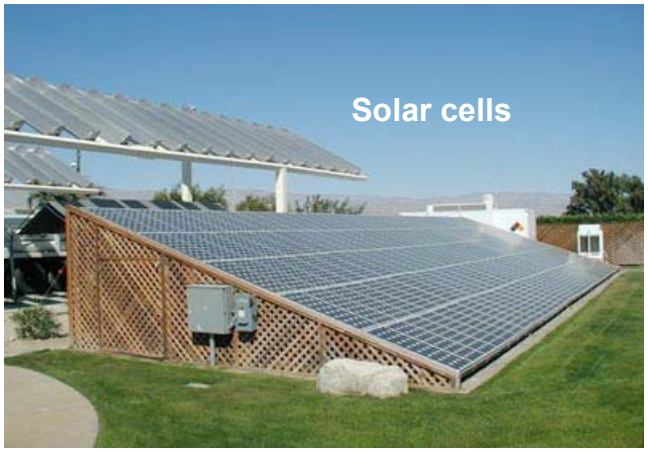


What can one do with it?

- Almost everything
- Material processing
  - Etching
  - Deposition
  - Cleaning
  - Sterilisation
- Light production



Semiconductor components


Solar cells


lamps

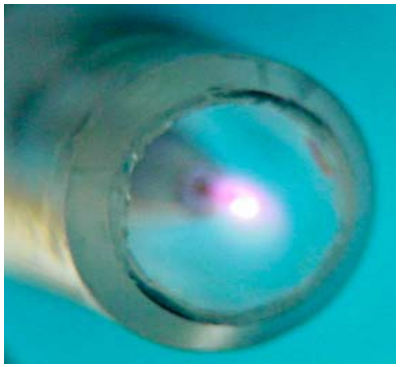
Living tissues?

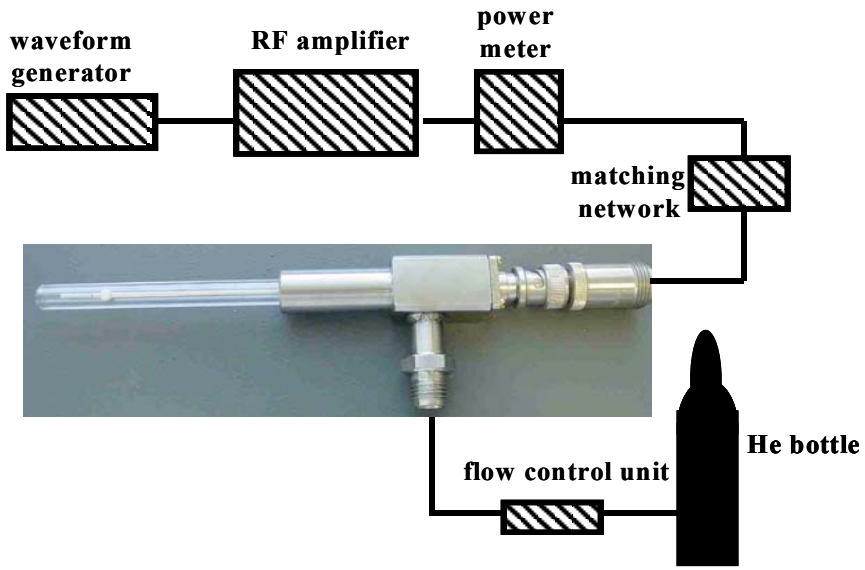
- Plasma can “clean” delicate objects
- What is more delicate than living organism?
- Problems…
  - High voltage
  - Temperature (*must* be below 40^{o}C !!!)
  - Radiation (UV damage)
  - Chemical damage
- … are solvable, but…

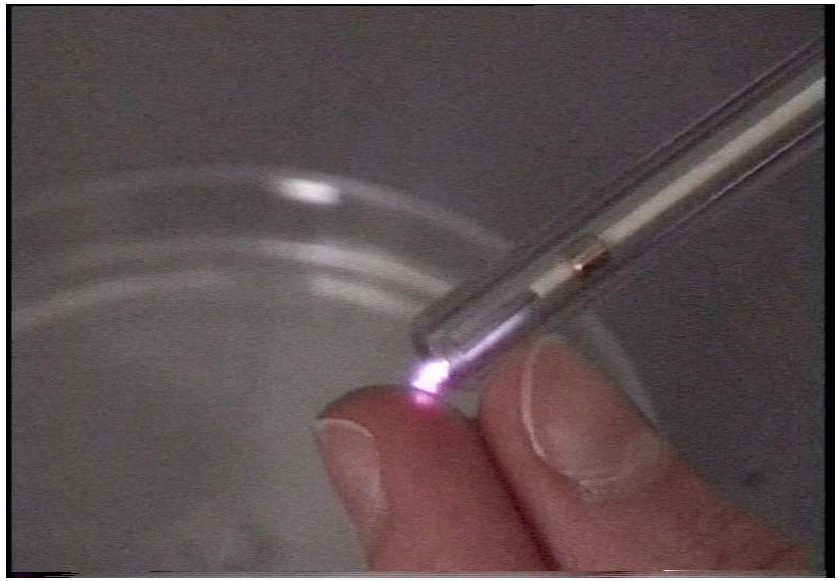
… a special source is needed!

Plasma needle

- RF-driven atmospheric source

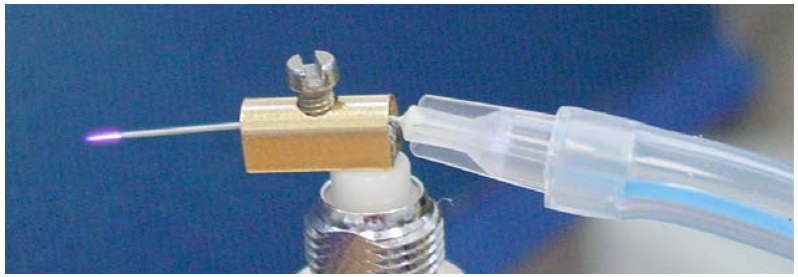




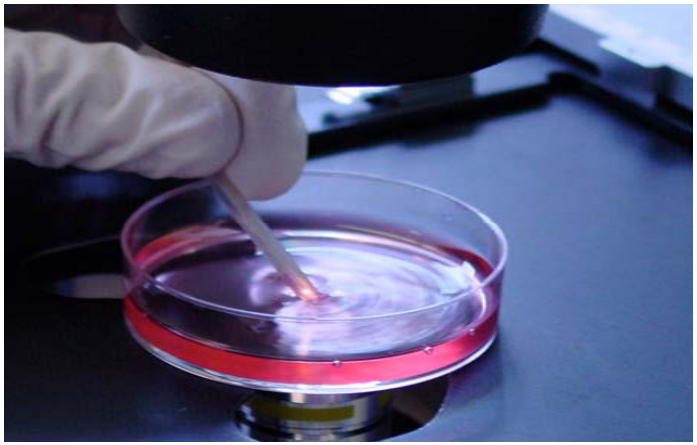


Fixed or flexible

- Fixed prototype: skin & dental applications



- Catheter: blood vessels



- Under liquid operation

Specifications

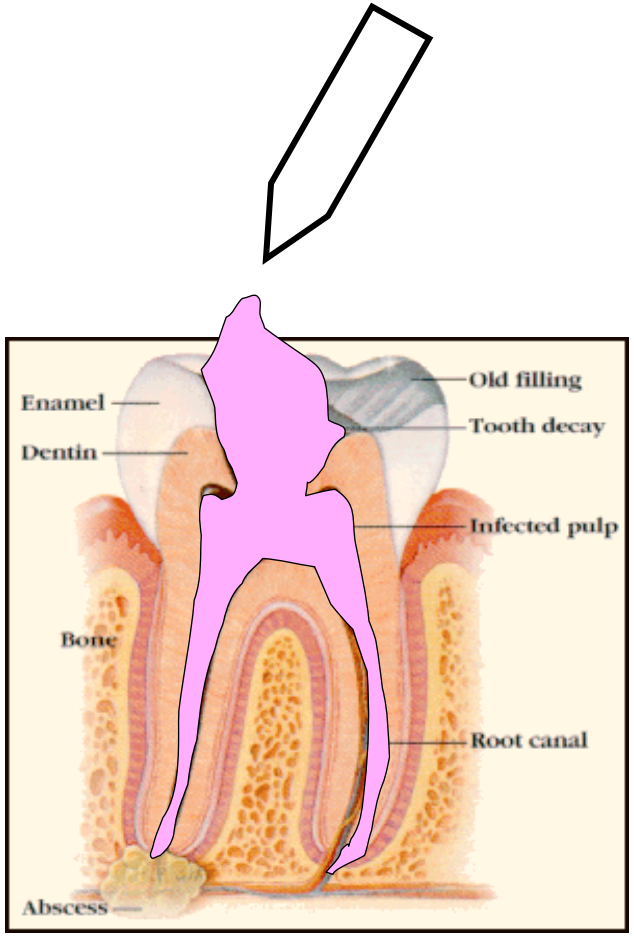
- Voltage < 400 V
  - RF does not disturb nerves/muscles
- Temperature < 60^{o} C (controllable)
- Very little UV radiation
- Charge density < 10^{17} m^{-3}
- Chemical species (radicals) < 10^{19} m^{-3}
- Resembles low-pressure plasma, but…
- Is atmospheric!

Medical applications

- Plasma treatment is:
  - Non-contact
  - Painless
  - Non-destructive (minimum damage)
  - Versatile!
- Killing bacteria *in vivo*:
  - Wound disinfection
  - Cleaning of dental cavities
- Cell and tissue modification
  - Cell removal (cancer)
  - Cell inactivation (cancer, stenoses, scars, etc.)
  - Cell activation (wound healing)

In vivo disinfection

- Gaseous medium: penetrates small fissures/cavities
- Tissue-saving treatment of caries
- Improvement of oral hygiene

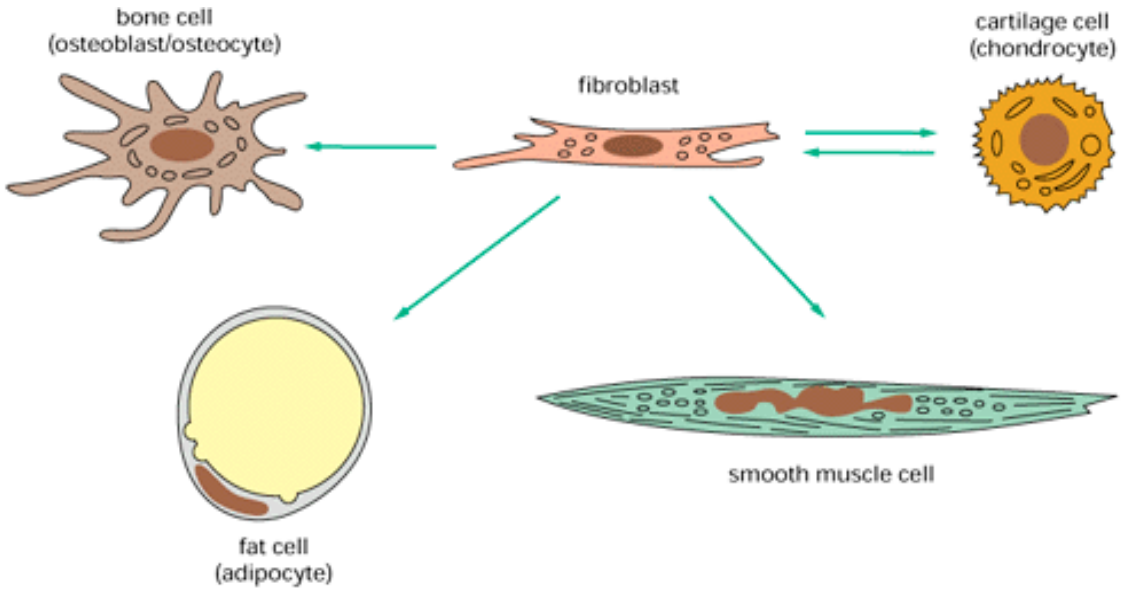


Various bacterial tests

- Thin biofilms < 0.1 mm: fast inactivation (seconds)
- Suspensions or thick biofilms 0.1-0.5 mm: slower (minutes)
- Very gentle conditions are sufficient (< 0.2 W)
- Safe & efficient

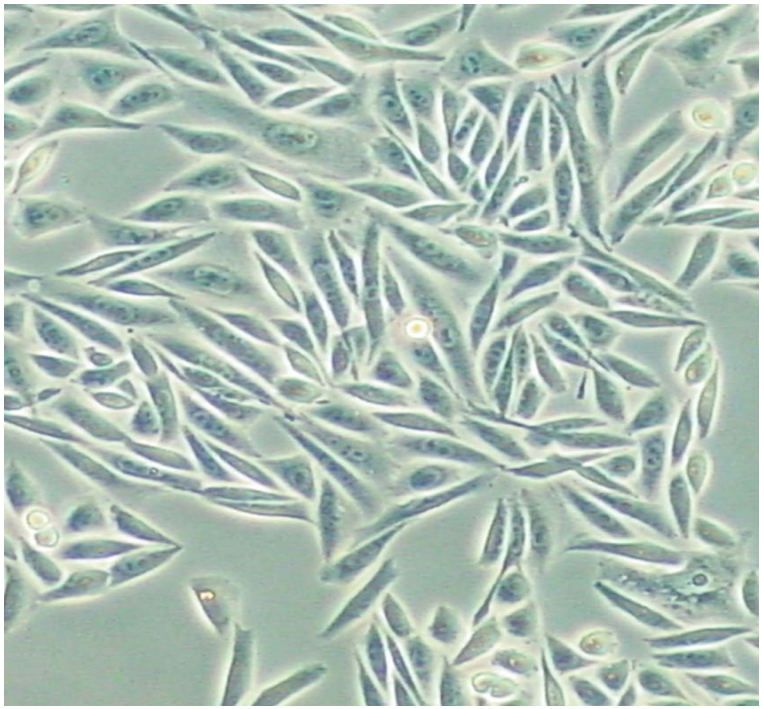
Study objects

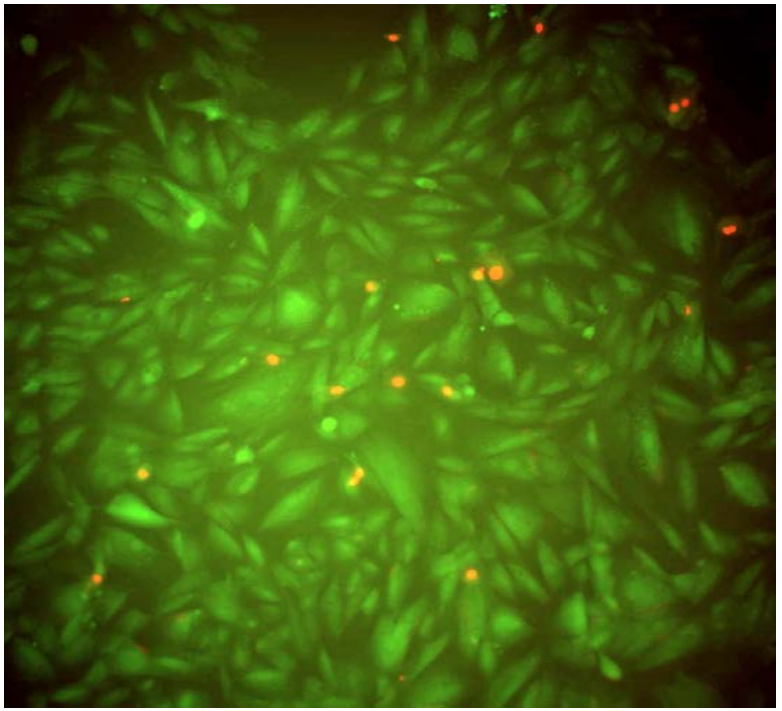
- Cells in culture
- Reproducible “2D tissue”
- fibroblasts (tissue repair!), arterial cells (cardiovascular obstructions!)



Cells in culture

- Attached to the scaffold & to each other by cell adhesion molecules (CAMs)



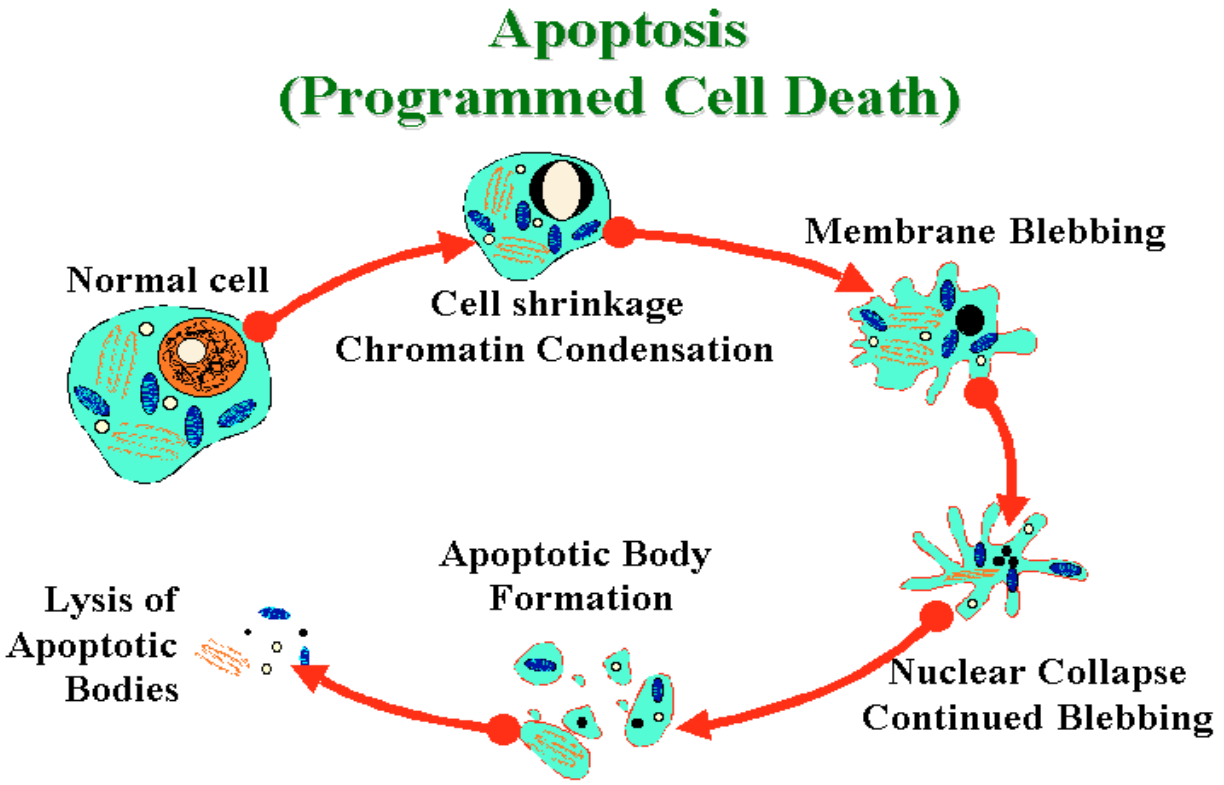


Cell & tissue treatment

- In conventional surgery:
  - Necrosis (acute cell death)
  - Inflammation
  - Scars
- “Operating without incision”
  - No necrosis
  - Tissue removal by means of programmed cell death (apoptosis)
  - No complications & scars

Apoptosis & necrosis

- Necrosis: membrane damage (leakage), tissue poisoning
- Apoptosis:

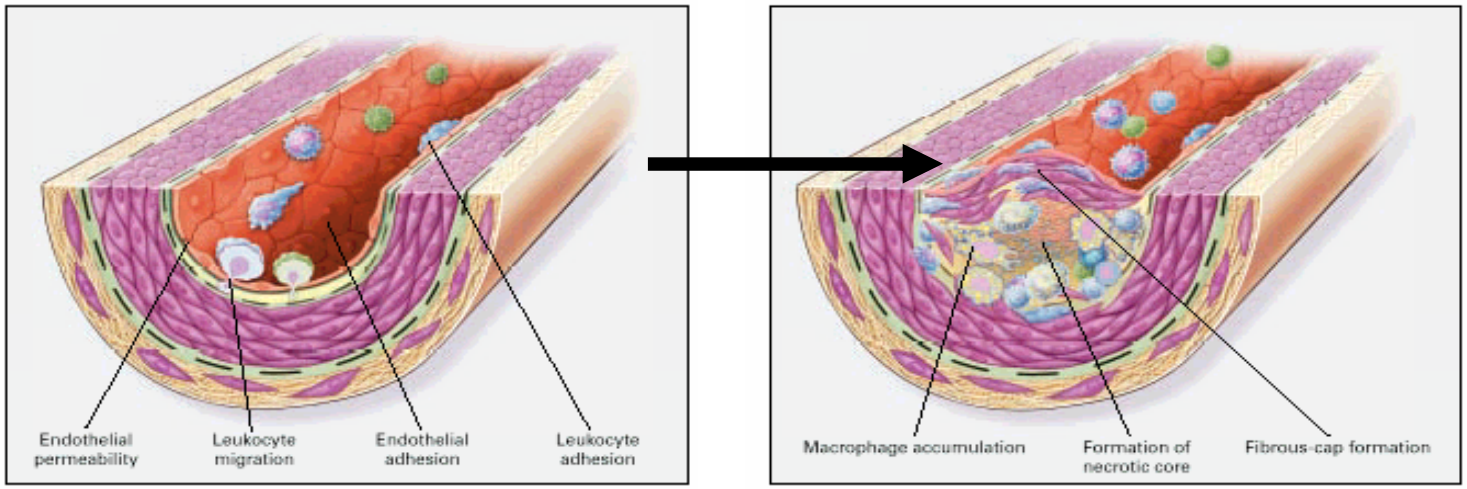


How to assay apoptosis?

- Many assays available (Annexin V, Caspase, M30 antibody), but…
- Visual observation works as well!
- Signs of apoptosis:
  - Early: DNA in nucleus condensed, membrane blebbing
  - Late: formation of apoptotic bodies, secondary necrosis

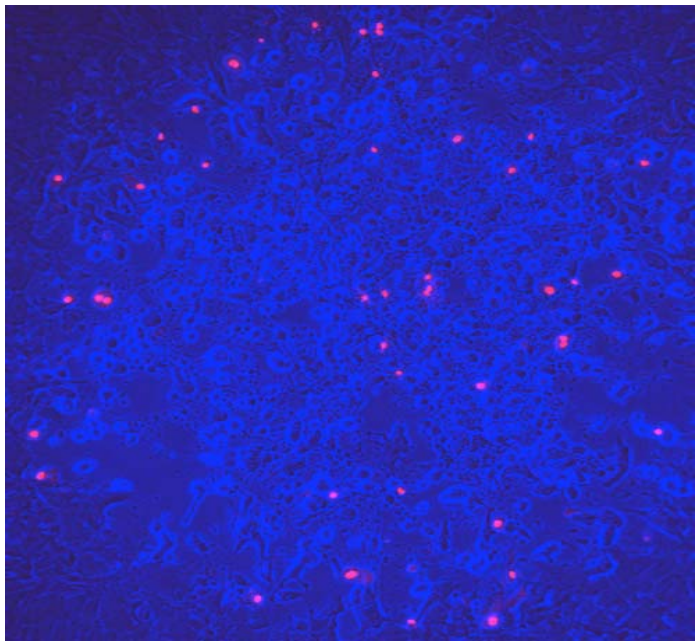
Arterial cells

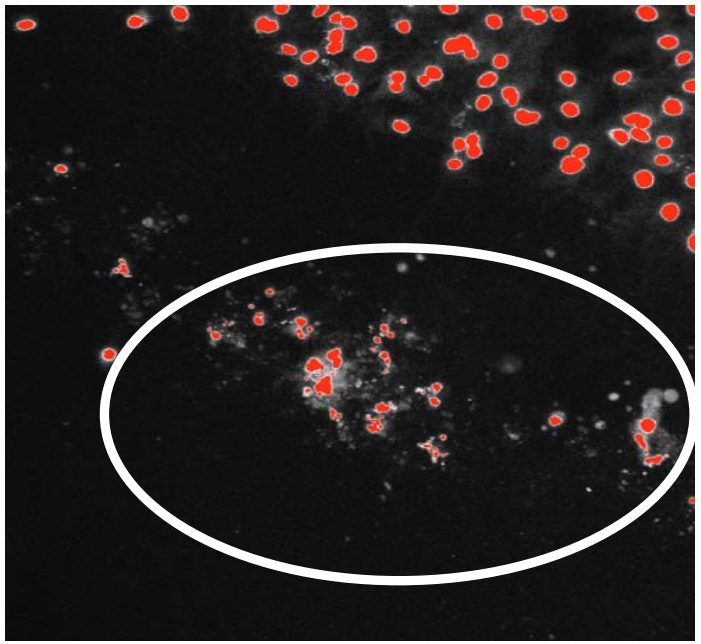
- Endothelial (intima, inside cell lining)
- Smooth muscle cells (media, intermediate layer)
- Stenosis (leads to heart infarct): overgrowth of media



Motivation in cardiovascular research

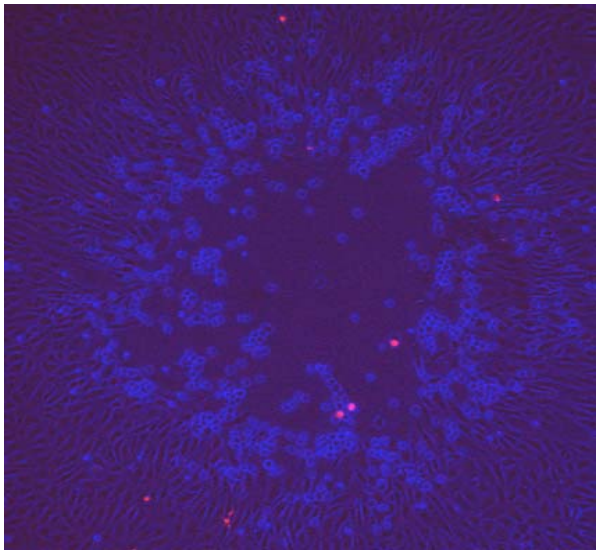
- Bring muscle cells (SMC) into apoptosis, or…
- Prevent them from proliferation
- With minimum damage to endothelial cells (EC)





Apoptosis

- Apoptosis in SMC works!
- Percentages > 50%
- Area of reach 0.5 mm to 1 cm
- Endothelial cells: no apoptosis, no necrosis
- Proliferation stop
- At 0.3-0.5 W

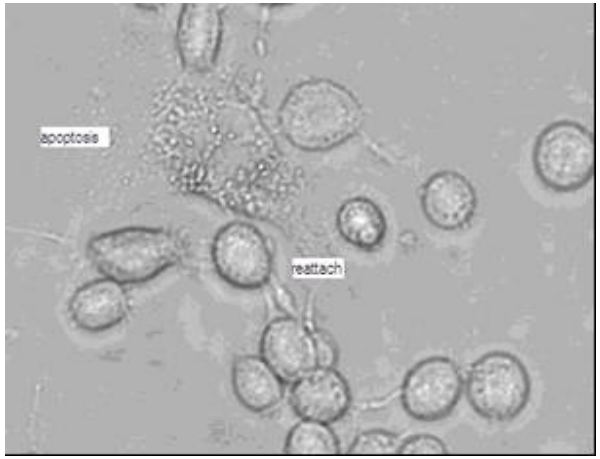


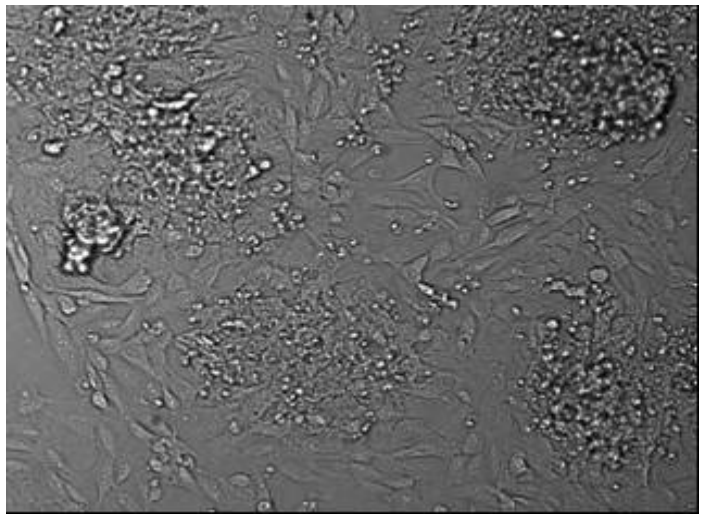
Perspectives

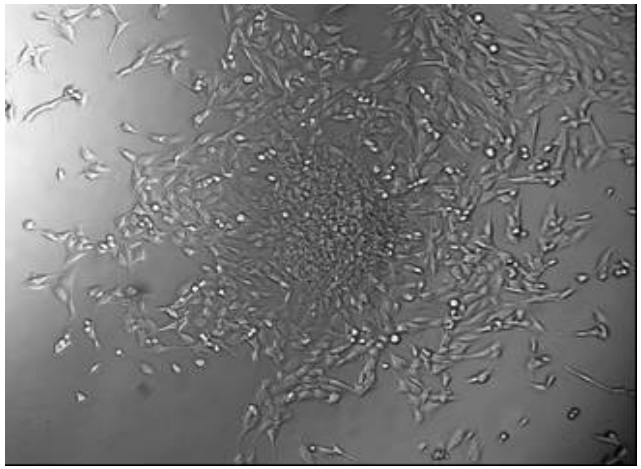
- SMC is more sensitive to plasma
- EC needs 2 x longer treatment
- Necrosis limited
- Dependent on dose
  - Apoptosis (SMC only)
  - Proliferation stop (both EC and SMC)
- Both effects OK!
- *In vivo* treatment feasible

Apoptosis in fibroblasts

- Apoptosis can be induced in many cell types
- Apoptotic bodies are “cleaned up” by remaining cells

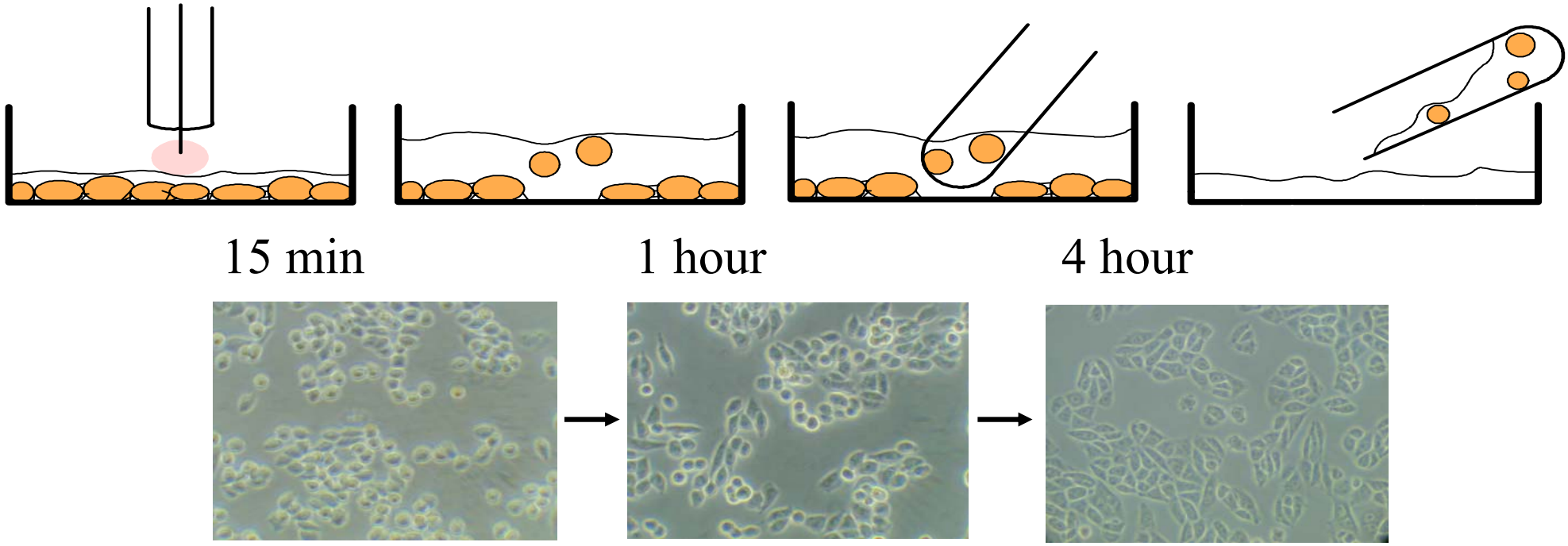






Other (sub-lethal) effects

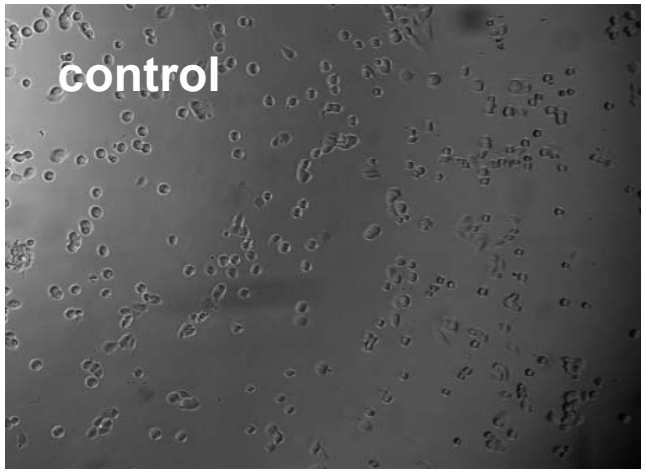
- Cell detachment at 0.1-0.2 W

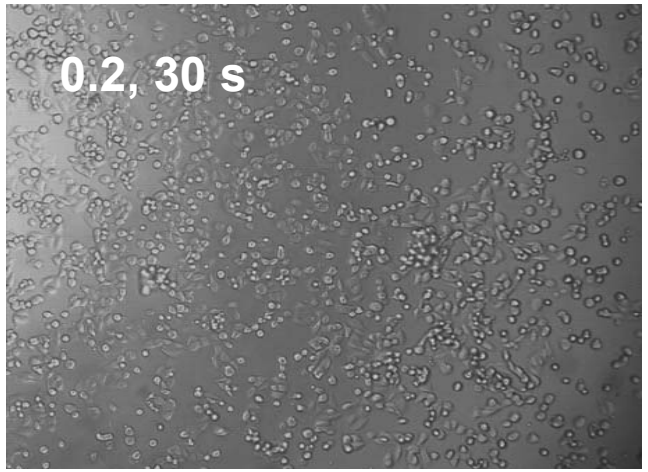


- Reversible cell extraction without damage!
- Making grafts?

Cell activation

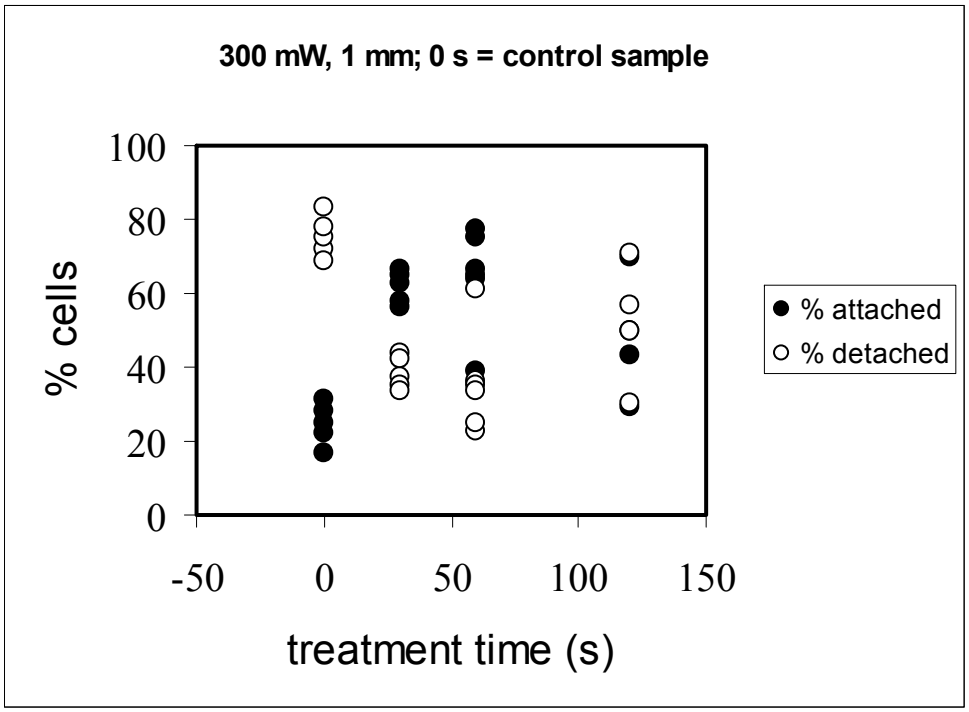
- Cells are treated in a suspension (plenty of liquid)
- Improved attachment and growth observed
- Liquid filters out damage factors, but a beneficial plasma species are still there!





Wound repair

- Great advantage in wound healing: disinfection *and* cell stimulation to repair the tissue!

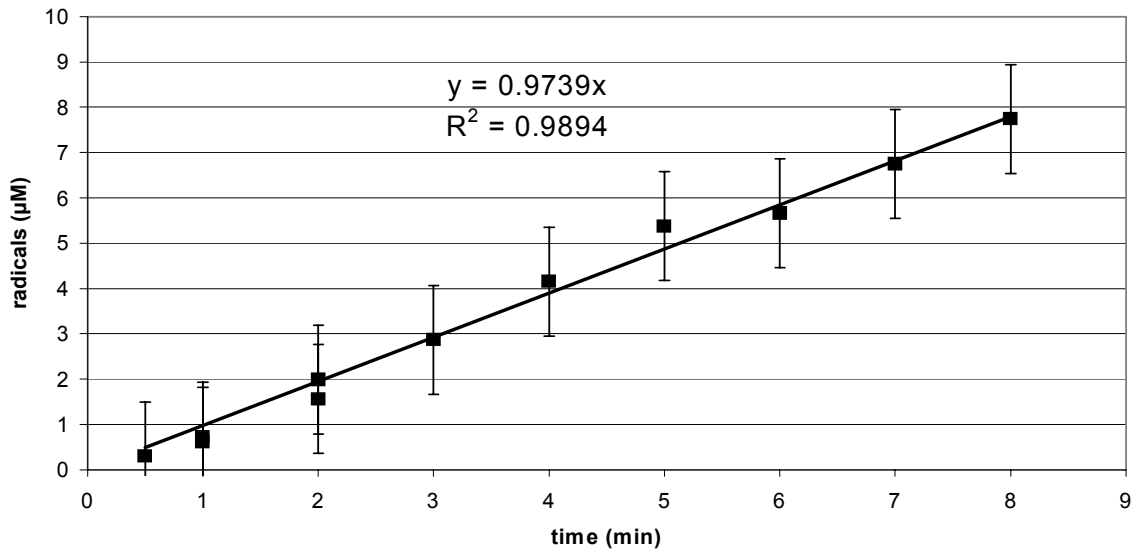


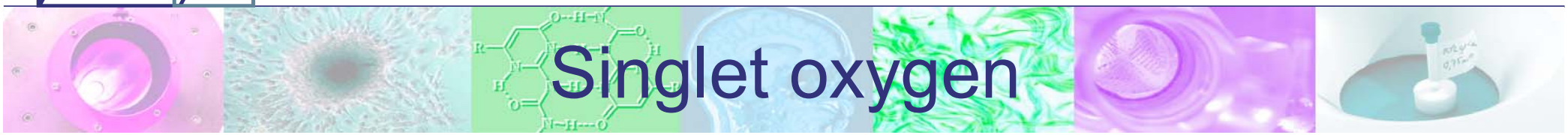
No magic: time for explanation!

- Reactive plasma species:
  - Ions – probably do not reach cells
  - Unstable, short living – radicals, helium metastables
  - Long living – singlet oxygen ($O_2(a)$)
- Effects:
  - Message of danger: detachment
  - Moderate damage: apoptosis
  - $O_2(a)$: increased metabolism?

Unstable radicals

- Reactive oxygen species (O, OH, etc.)
- Aggressive damage factors, but…
- Densities are very low (physiological range) and controllable
- Plasma supplies radicals to the sick section
- The work is done in a natural way!





Singlet oxygen

- 1 eV more energy than ground state
- All reactions are faster, thus also glucose production
- Can be used in energy (ATP) production
- Gives cells “energy boost”

Summarising:

- Some problems had to be solved,
- … but it works!
- Cold plasma technology is versatile…
- … from external disinfections to catheter operations
- Will appear in dentistry, skin surgery, cardiology, cell manipulation, etc.
- … and motivate & stimulate fundamental plasma & biology research.