AUTUMN COLLEGE ON PLASMA PHYSICS
5 - 30 September 2005

Cold gas plasma in medicine and biology

Eva Stoffels
Eindhoven University of Technology, the Netherlands
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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
Plasma can “clean” delicate objects
What is more delicate than living organism?
Problems…
  – High voltage
  – Temperature (must be below 40°C !!!)
  – Radiation (UV damage)
  – Chemical damage
… are solvable, but…
... a special source is needed!
Plasma needle

• RF-driven atmospheric source
• Fixed prototype: skin & dental applications

• Catheter: blood vessels

• Under liquid operation
Specifications

- Voltage < 400 V
  - RF does not disturb nerves/muscles
- Temperature < 60° 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**: (Programmed Cell Death)

  - Normal cell
  - Cell shrinkage Chromatin Condensation
  - Membrane Blebbing
  - Lysis of Apoptotic Bodies
  - Apoptotic Body Formation
  - Nuclear Collapse Continued Blebbing
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 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
• 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?

15 min 1 hour 4 hour
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!

(control) 0.2, 30 s
Wound repair

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

![Graph showing % cells vs. treatment time (s) for 300 mW, 1 mm; 0 s = control sample.](image)
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!

![Graph showing the relationship between time and radicals](image)

\[ y = 0.9739x \]

\[ R^2 = 0.9894 \]
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.