
Atmospheric Plasma in Medicine: Chronic Wound Disinfection

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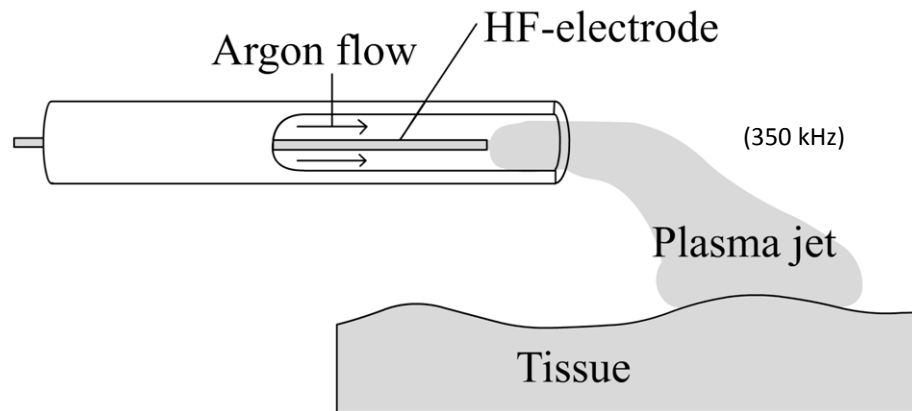
Outline

- Introduction (history and possible application of plasma medicine)
- Wound disinfection: clinical study
- Summary

Atmospheric plasma for medical use: history

- 1900s~ :1st generation
mainly heat is used for tissue removal
(electric scalpel, etc.)
- 1970s~ :2nd generation
thermal energy is used for superficial treatment
(argon plasma coagulator)
- 1990s~ :3rd generation
contact-free superficial treatment
charged particles, reactive species, UV photons,
electric field play a role

Argon plasma coagulator (ERBE GmbH)



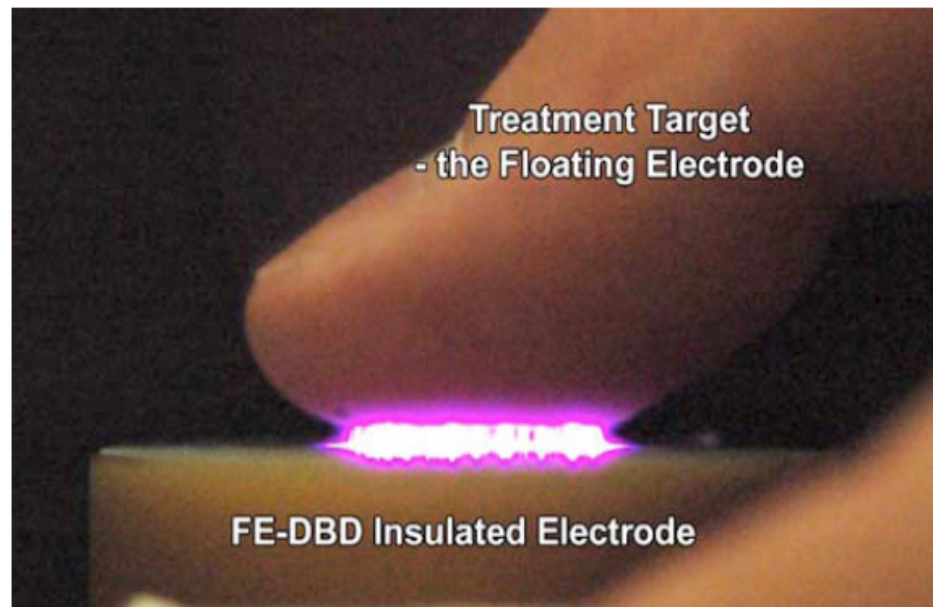
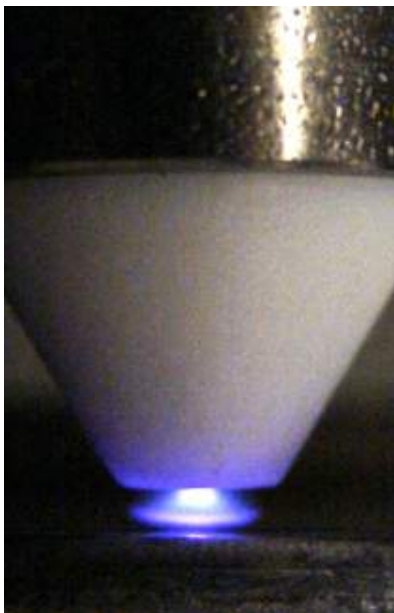
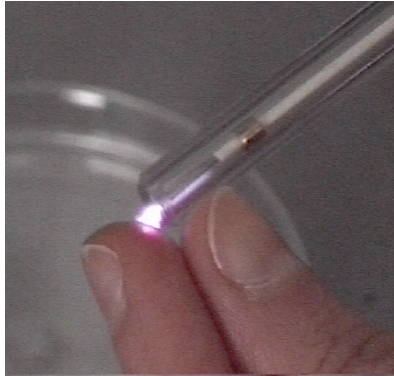
Argon plasma coagulation (APC) is a medical endoscopic procedure used primarily to control bleeding from certain lesions in the gastrointestinal tract, and also sometimes to debulk tumours in the case of patients for whom surgery is not recommended. It is administered during esophagogastroduodenoscopy or colonoscopy.

*http://en.wikipedia.org/wiki/Argon_plasma_coagulation
<http://www.erbe-med.de/index.html>

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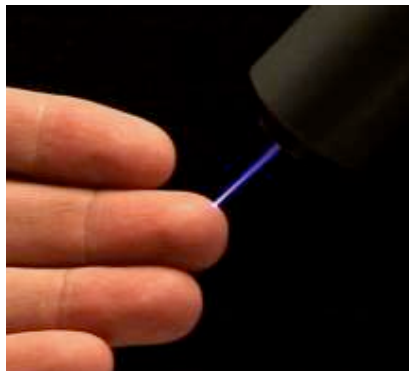
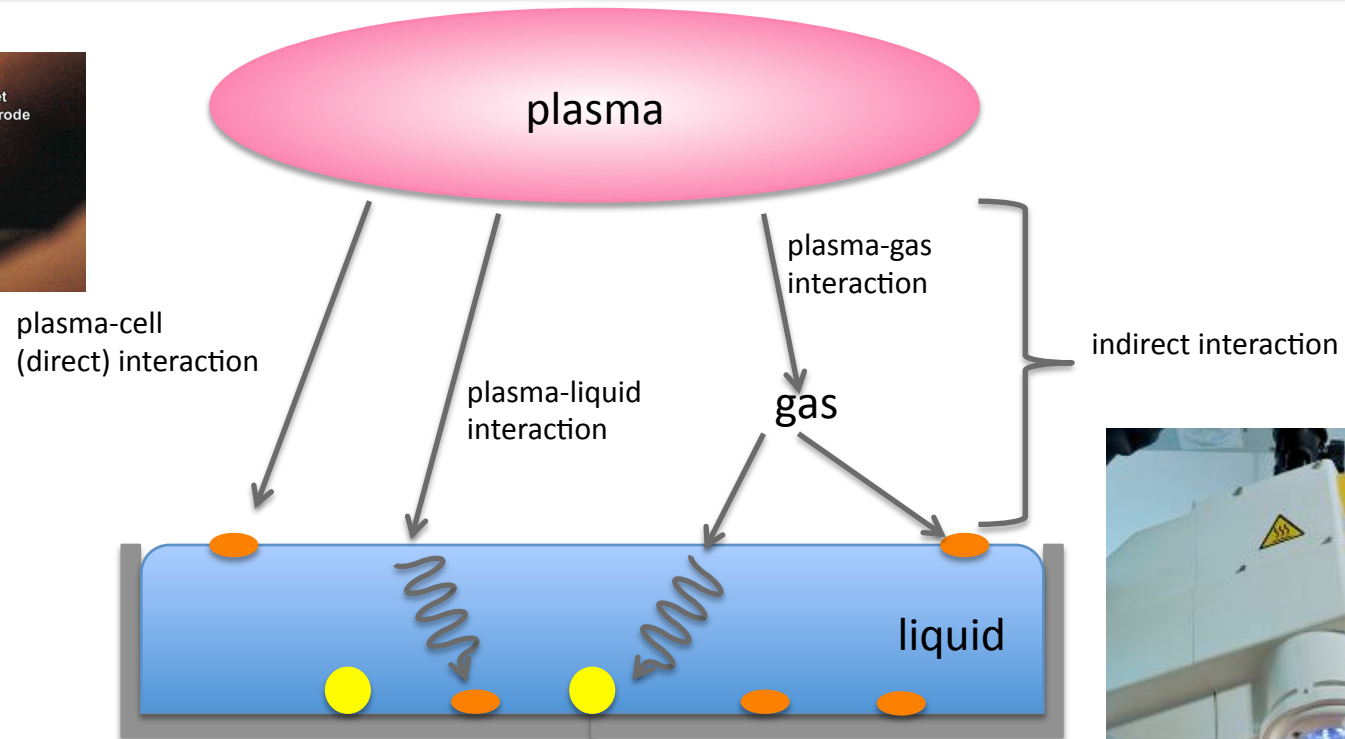
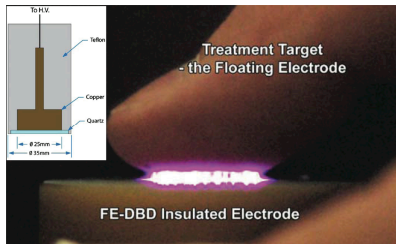
Plasma devices



What plasma can do in medicine

- Sterilization/ disinfection
(bacteria, fungi, virus)
- Blood coagulation
- Triggering of apoptosis on cells
- Improvement of proliferation of human cells
- Teeth bleaching, etc.

Interaction of plasma with living tissues

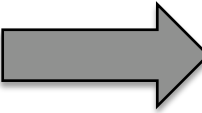


cell membrane permeabilisation
and reactive species transport




Agents from plasma

Electrons (1-10 eV)
Ions (~1 eV)
Photons (UVC: 4-10 eV)




Bond breaking (DNA)
Physical sputtering

Reactive oxygen and
nitrogen species (ROS, RNS)



Oxidative stress
Proliferation (NO, etc.)

Electric field
(external and local charging)



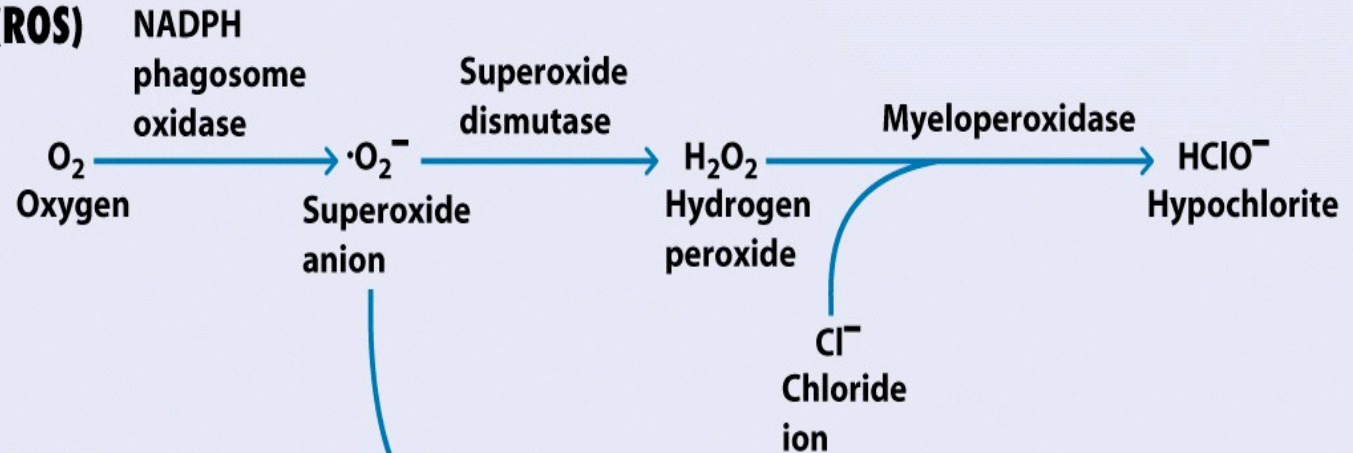
Membrane disruption
stimulation

ROS and RNS

Antimicrobial species generated from oxygen and nitrogen

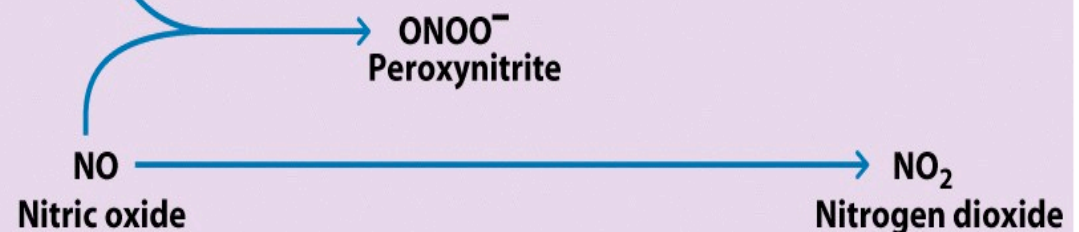
Reactive oxygen species (ROS)

$\cdot\text{O}_2^-$ (superoxide anion)
 $\text{OH}\cdot$ (hydroxyl radical)
 H_2O_2 (hydrogen peroxide)
 ClO^- (hypochlorite anion)



Reactive nitrogen species (RNS)

NO (nitric oxide)
 NO_2 (nitrogen dioxide)
 ONOO^- (peroxynitrite)

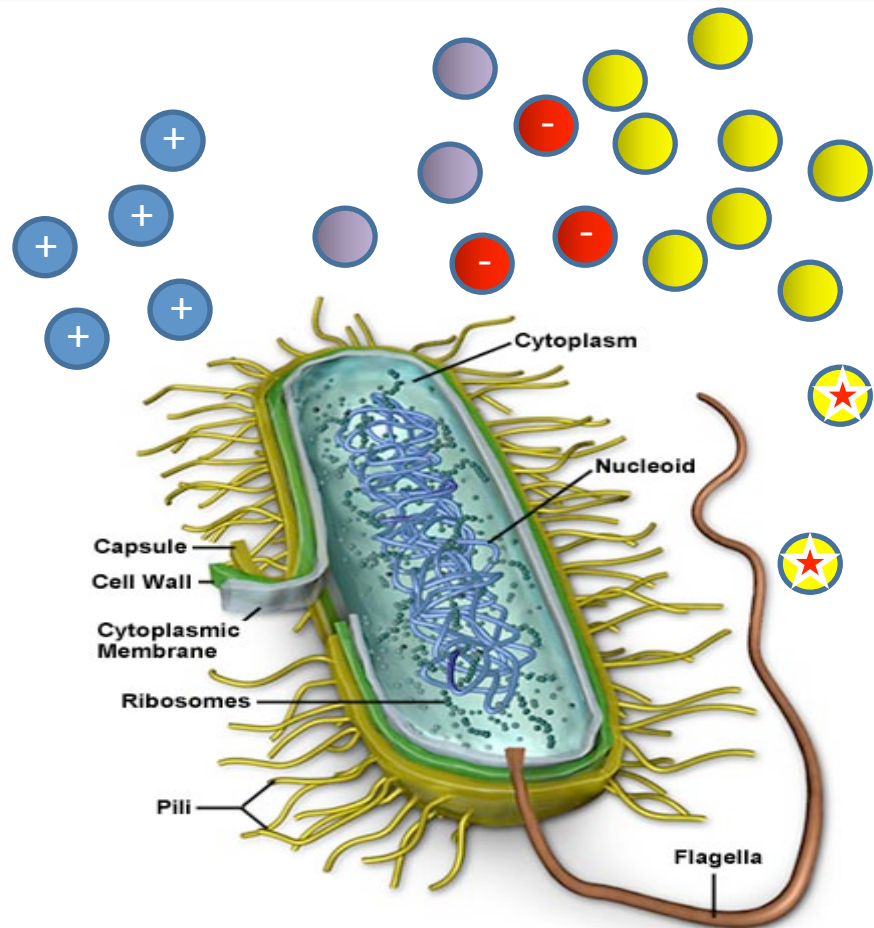


T. J. Kindt et al, *Kuby Immunology* 6th edition (W. H. Freeman, 2006).

Cell permeabilization

Unique Plasma Features

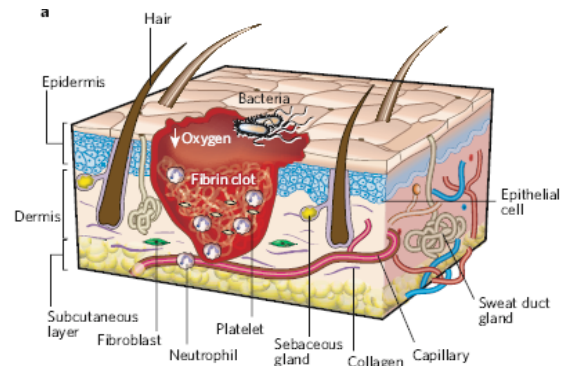
- Electron-ion recombination can make the bacteria cell wall permeable (up to 5 nm).
- Non-equilibrium chemistry produces many **reactive O, H and N species**, e.g. NO, NO₂, •OH, O₂⁻ and H₂O₂, which can then penetrate
- **Bacterial DNA in the cytoplasm** is directly accessible to the reactive species.



<http://micro.magnet.fsu.edu/cells/bacteriacell.html>

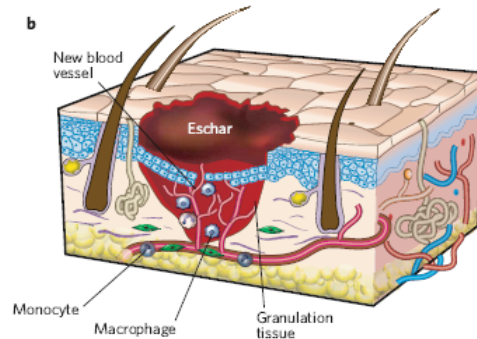
Yonson et al., *J. Phys. D* (2006), Halliwell and Gutteridge, Oxford Univ. Press (2007), Stoffels et al., *IEEE Trans. Plasma Sci.* (2008), Nosenko et al., *NJP* (2009), Leduc et al., *NJP* (2010), Liburdy and Vanek, *Rad. Res.* (1985)

Possible medical applications: wound treatment



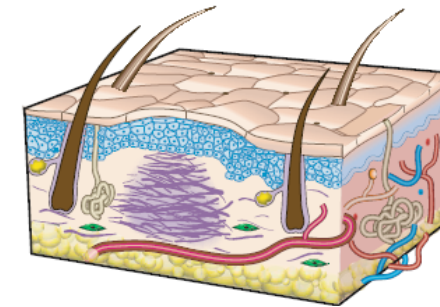
Inflammatory phase

- ~48 hours
- bacteria sterilization
- blood coagulation



Proliferative phase

- 2~10 days
- (blood vessels generation)
- collagen deposition from fibroblasts



Remodeling phase

- 1 year
- tissue reorganization
- apoptosis of unnecessary cells

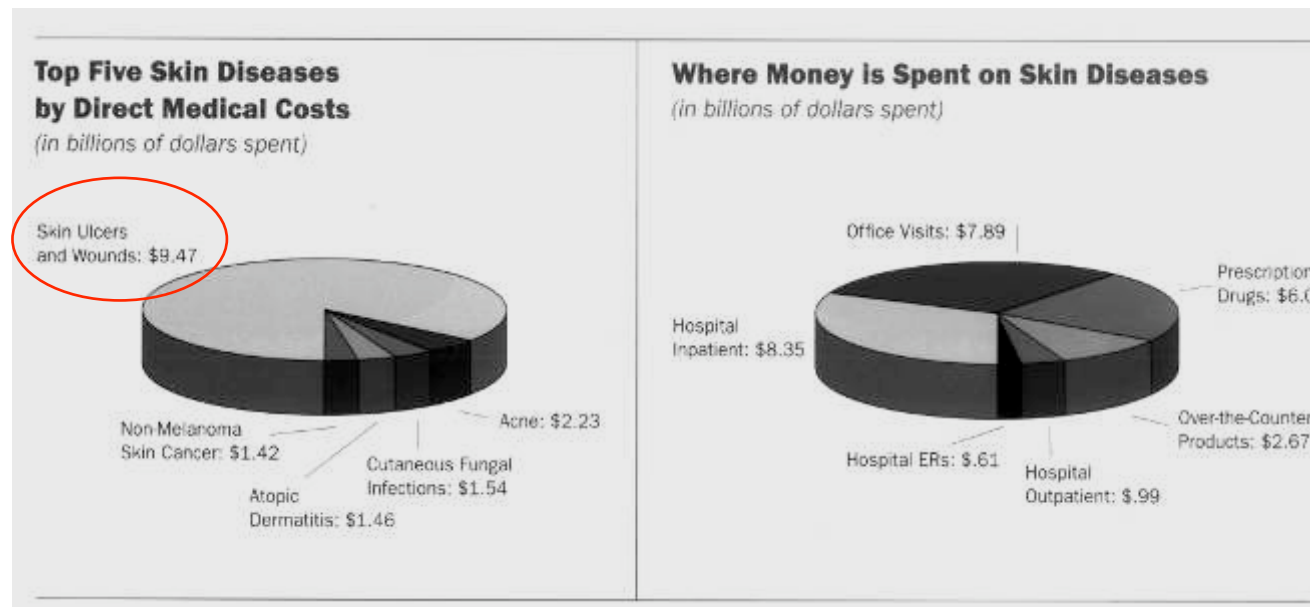
R. A. Bryant, et al., *Acute and Chronic Wounds* (Mosby, Missouri, 2006).

G. C. Gurtner., *Nature* **453** (2008) 314.

Chronic wounds

Chronic wounds are a major burden for the health system

- Prevalence ~ 1-2 % in German Population (> 1.000.000 patients)
- High costs for the community 1-2 % of annual health care budget*
- Venous ulcers require an average of 24 weeks to heal, 15% never heal, recurrence is found once or multiple times in 15-71% of cases** ***



American Academy of Dermatology Report 2005

*Etufugh CN, Phillips TJ. Venous ulcers. *Clin Dermatol* 2007; **25**: 121-30.

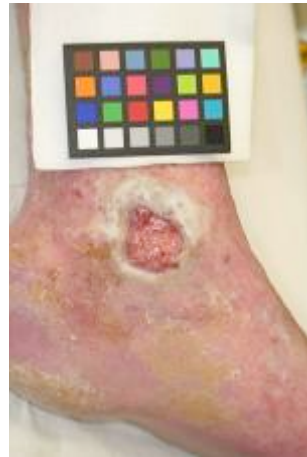
**Kurz et al. VEINES Task Force Report, *Int Angiol*. 1999;18(2):83-102.

***Heit et al. Venous thromboembolism epidemiology *Semin Thromb Hemost*. 2002;28(suppl 2):3-13

Chronic wounds in dermatology



Venous diseases



Arterial diseases



Infections



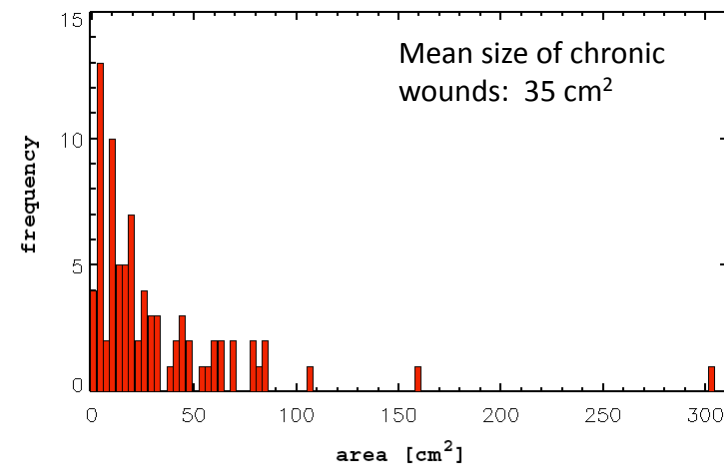
Diabetes mellitus



Carcinoma



Pyoderma gangraenosum



Infections and resistance: facts

- „Bacteria can become resistant to antibiotics“ warned Alexander Fleming, when he landed the Nobel prize in Medicine in 1945.
- European Antimicrobial Resistance Surveillance System (EARSS) 2007: Resistance is becoming a larger problem year after year (especially for *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Escherichia Coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*)
- Global Health Care Associations consider multi-resistant germs like MRSA as a global threat*
- 19,5 % of all *Staphylococcus aureus* detected in German hospitals are MRSA (EARSS 2008)
- Worrying is the raising resistance against so called reserve drugs within the last 6 years – e.g. Vancomycin (EARSS 2007)
- November 2008 launch of DART (Deutschen Antibiotika-Resistenzstrategie)

*Grundmann H, Aires-de-Sousa M, Boyce J et al. Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public-health threat. *Lancet* 2006; **368**: 874-85.

Liquids for wound treatment

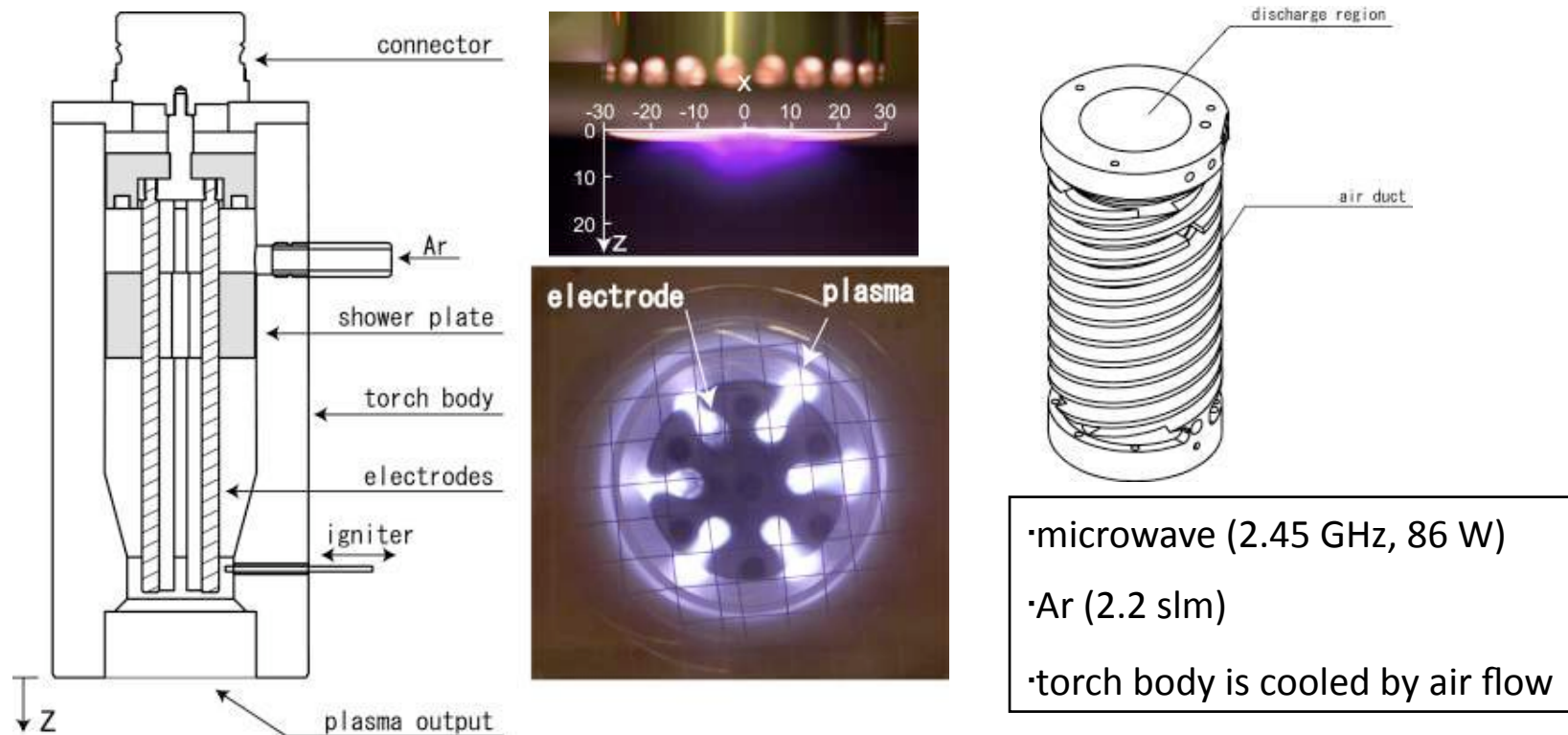


Phase I study

Studies conducted:

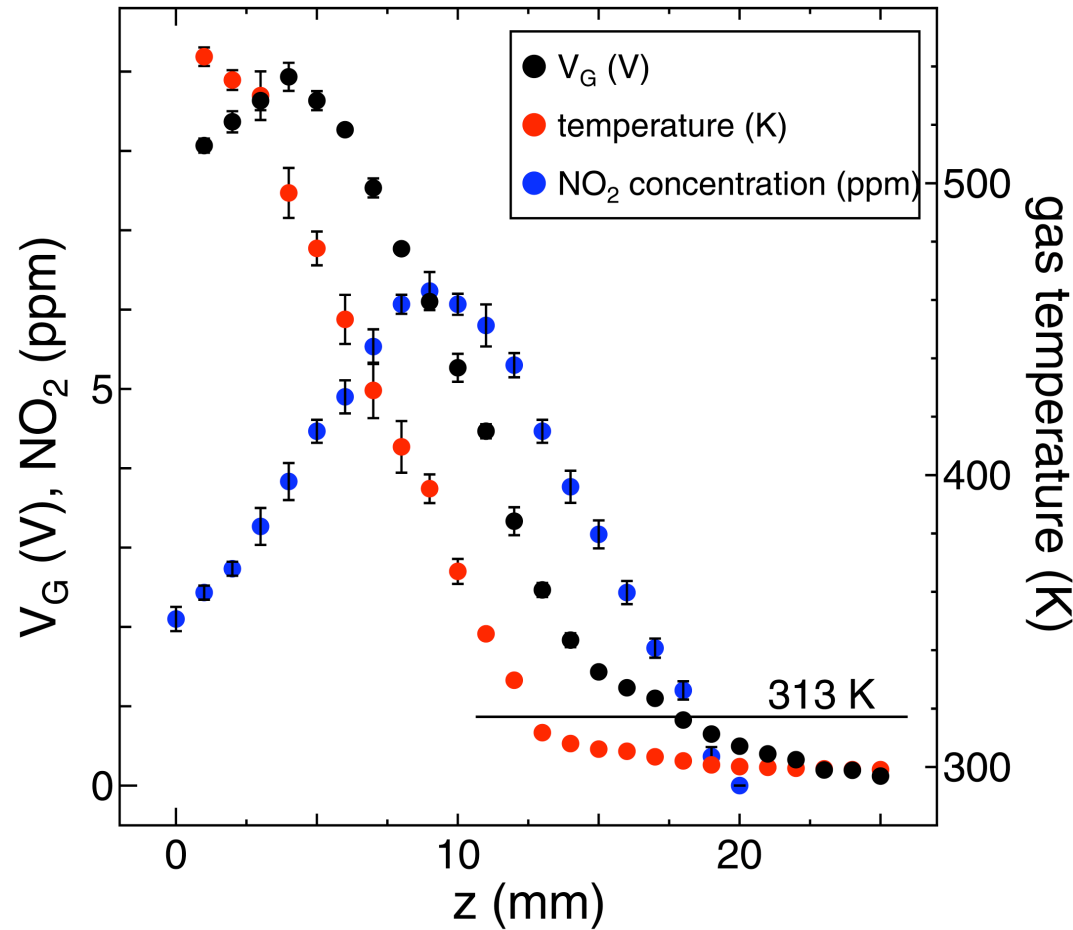
- Demonstration of bactericidal effect of plasmas (gram positive and gram negative)
- Demonstration that bacteria of relevance for wound healing are killed (incl. fungi)
- Demonstration that the plasma-effect is not temporary
- Demonstration that gas-temperature (lower than 50° C) has no bactericidal effect
- Demonstration that there is no thermal damage to living tissue
- Demonstration that surviving bacteria do not develop „plasma-resistance“ (1 Iteration)
- Demonstration that surviving bacteria do not develop resistance to antibiotics
- Demonstration that surviving bacteria do not develop altered biochemical properties
- Demonstration that human blood is not altered during plasma treatment
- Demonstration that the histology of treated human skin is unaltered (treatment < 10 min)
- Demonstration that there are no morphological changes in HeLa-cells

Plasma torch



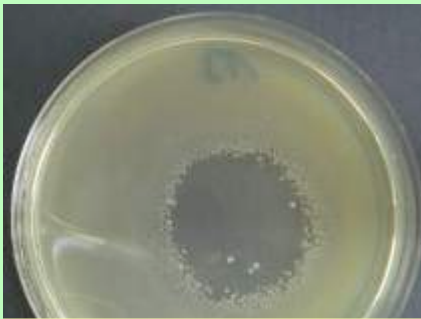
The plasma torch consists of 6 stainless steel electrodes placed inside an aluminium cylinder of 135 mm in length. The centers of the 6 electrodes, whose surfaces are serrated, are distributed equally at a distance of 6 mm from the inner surface of the cylinder. 6 small plasmas are produced between each of the electrode's tips and the inner surface of the cylinder as shown in the figure.

Plasma characteristics



Around $z = 7 - 13$ mm, the gas temperature decreases as well as the floating potential of the mesh grid decreases. The NO_2 concentration profile has the highest value. This suggests that the charged particles and gas flow from the torch has a contact with the ambient air.

Bacteria relevant to chronic wounds



Escherichia coli



Group A streptococcus



*methicillin-resistant
Staphylococcus aureus*



Burkholderia cepacia



Enterococcus faecalis



*vancomycin-resistant
Enterococcus faecium*



*Pseudomonas
aeruginosa*



Bacillus cereus

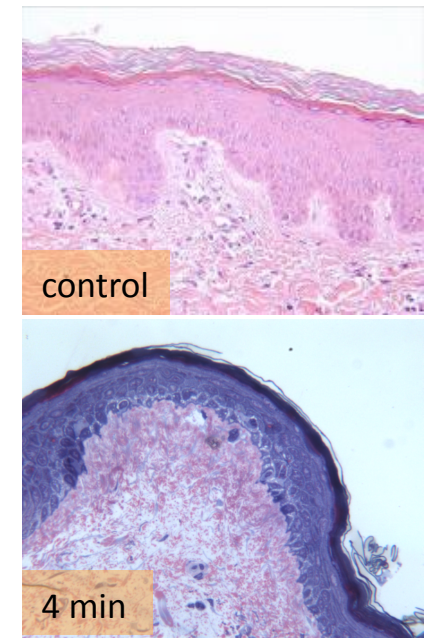
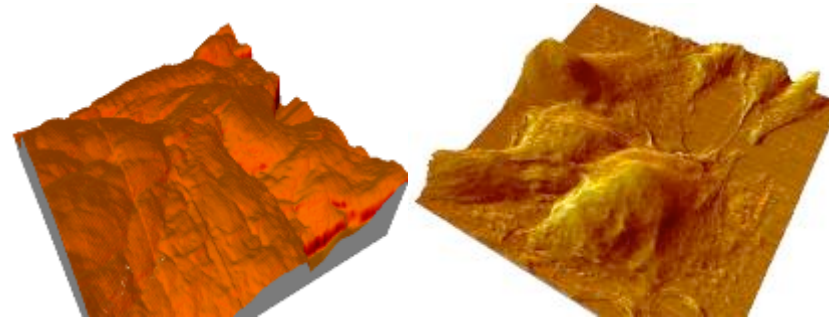
present on healthy persons

facultative pathogenic,
occasional resistance

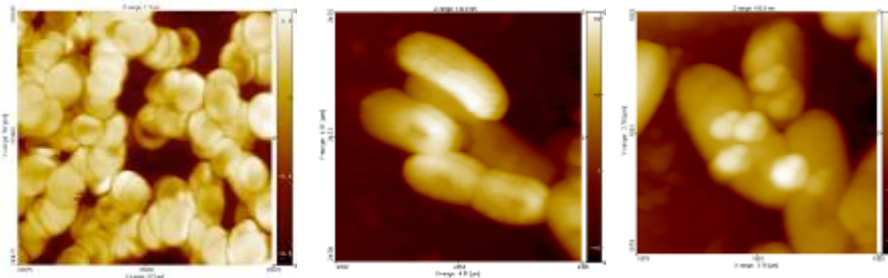
facultative pathogenic, seldom present on healthy skin

Safety of plasma: Phase I study

Numerous tests to find dosages and to check harmlessness of the plasma treatment:
e.g. histologies, blood tests, microscopic images, AFM, cell assays...



Further investigations with fibroblasts, keratinocytes, cell cultures, essays to check toxicity, mutagenicity, and antibodies



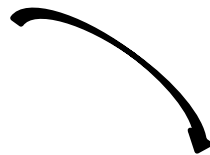
Acceptance procedure: Phase II study

Ethics commission:

- Proposal
- Study Protocol incl. Case Report Forms
- Compliance with EU medical device Directive (MDD)
 - Council Directive 93/42/EEC
 - Medical Device Directive MDD
- Patient Insurance (Gerling Versicherungen)

DIMDI:

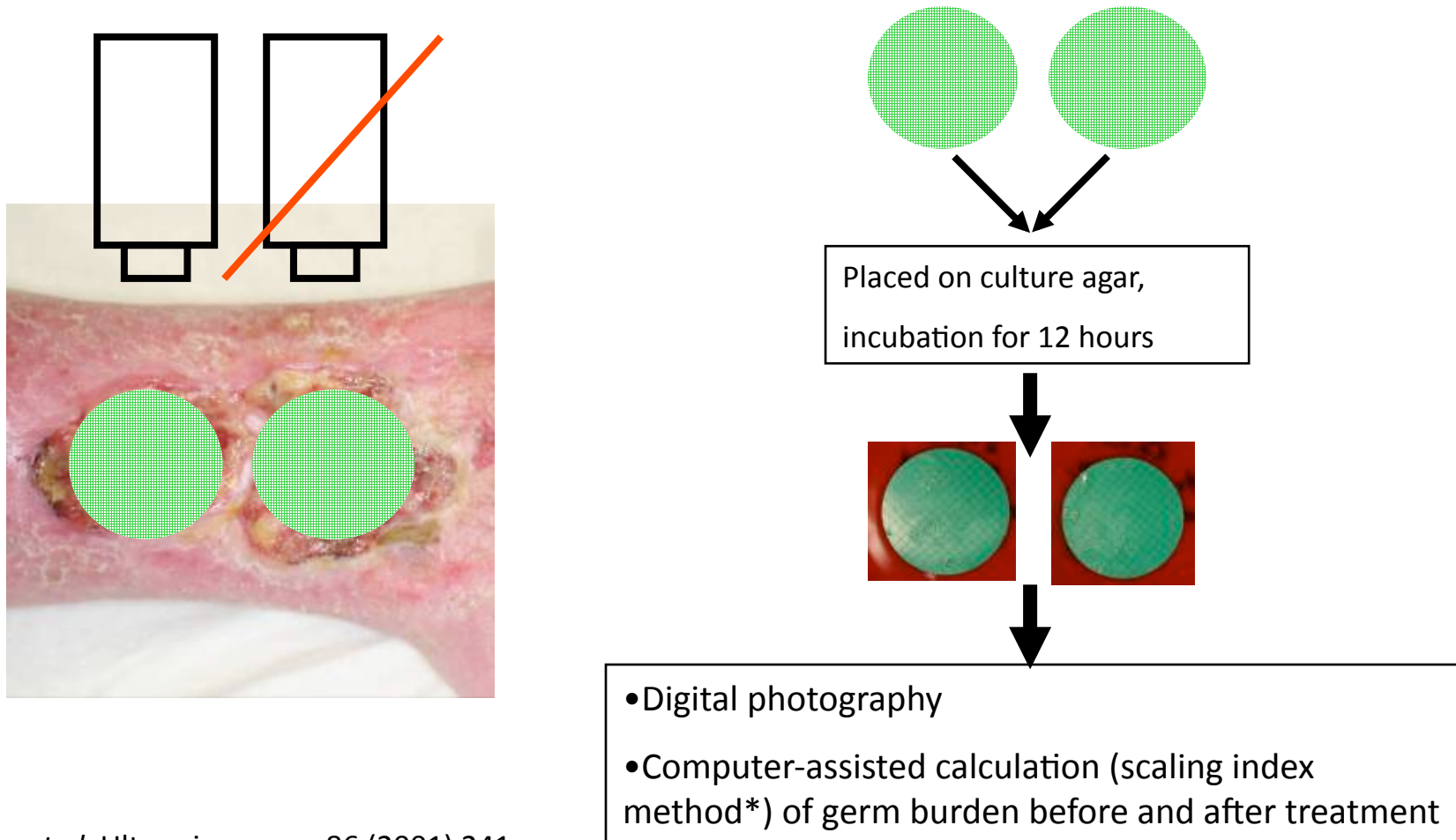
- Registration No: DE/CA126/AP4/3332/12/05
- Description: STERILISATOR



Technical Standards MicroPlaSter:

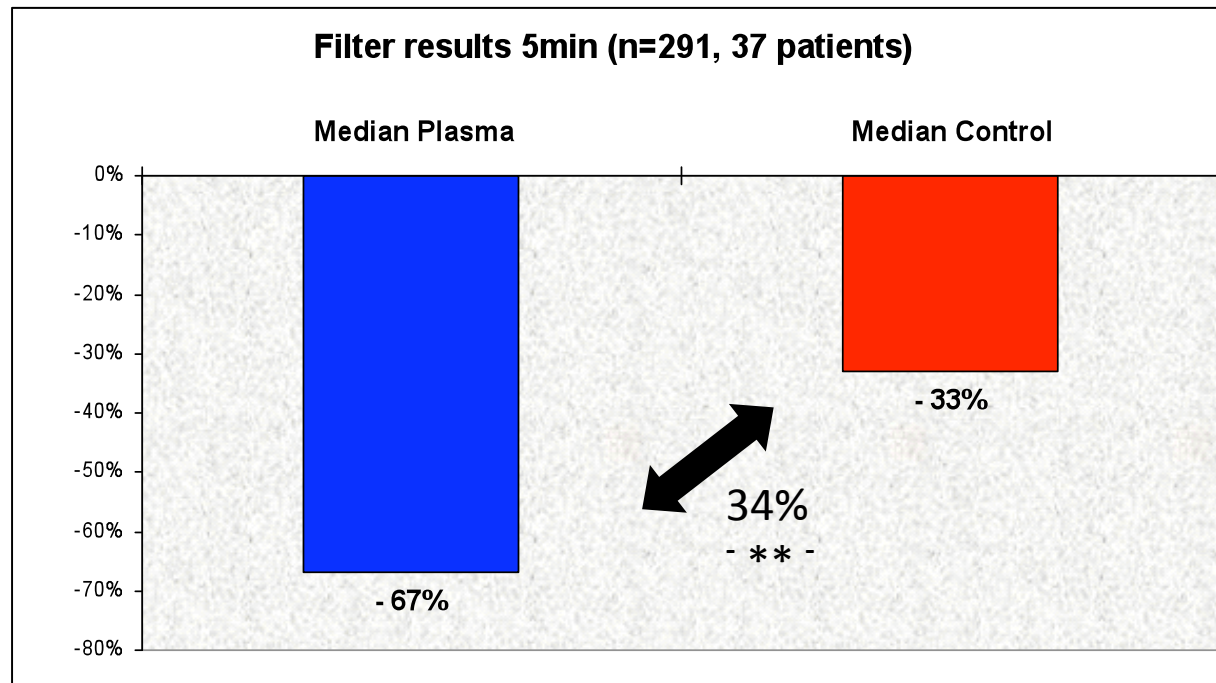
- Proof of technical safety :
 - General Requirements for Safety (EN60601-1)
 - Safety Requirements for Medical Electrical Systems (EN60601-1-1)
 - Electromagnetic compatibility (EN60601-1-2)
- User Manual and User Maintenance Manual
- Risk valuation according to EN ISO 14971
- Additional Standards:
 - Graphical symbols for use in the labelling of medical devices (EN980)
 - Information supplied by manufacturer with medical devices (EN1041)

Evaluation of bacteria load



*F. Jamitzky *et al*, Ultramicroscopy 86 (2001) 241.
G. Morfill *et al*, Physics World (2000) 41.

Results: with 5 min treatment

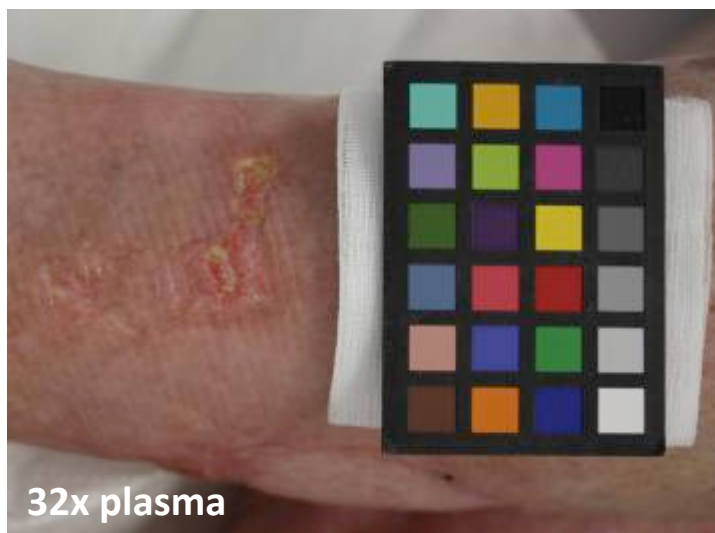
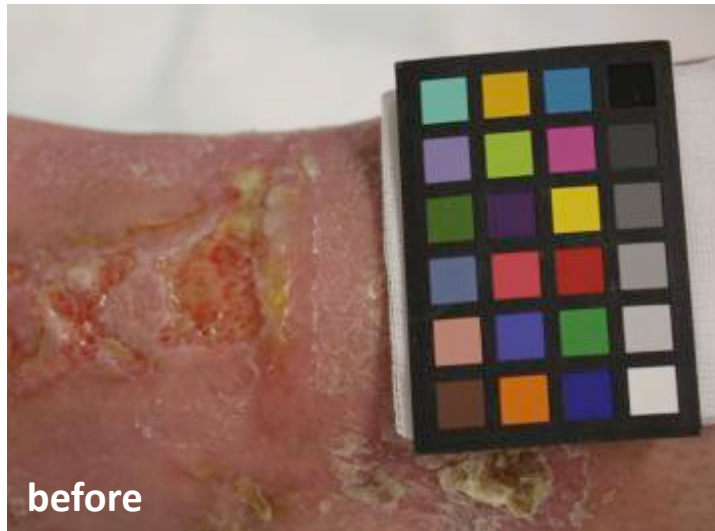


37 patients
291 treatments
5 min treatment time

Primary aetiology of
wounds: venous ulcers
(47%)

Highly significant ($p < 10^{-6}$) higher germ reduction (34%) in plasma treated area

Good results of plasma therapy



Insufficient results of plasma therapy



Summary

Atmospheric plasmas can be effective in medicine. In our group, the bactericidal property of plasma has been shown *in vivo*. To improve the characteristics of plasmas, the followings are necessary:

Understanding the **physics** of cold atmospheric plasmas

Understanding the **chemistry** of cold atmospheric plasmas

Understanding the **transport** from cold atmospheric plasmas

Understanding **plasma-cell/protein** interaction through gas/liquid

Designing **plasmas for hygiene**

Designing **plasmas for medicine**

are necessary.



plasma physics
engineering
plasma chemistry
fluid dynamics
biology
microbiology
cell biology
medicine

acknowledgement



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