NEW ACHIEVEMENTS OF PLASMA APPLICATIONS IN MEDICINE

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LAYOUT

- Objectives
- Introduction
- What is Plasma?
- Atmospheric-pressure plasmas (cap)
- Scientific basis of modern plasma medicine
- Atmospheric-pressure plasmas for medical purposes
- Therapeutic applications of cap

OBJECTIVES

- The field of plasma applications for the treatment of medical materials or devices is intensively researched and partially well established for several years. Plasma medicine is an innovative and emerging field combining plasma physics, life science and clinical medicine.
- Plasma medicine in the sense of its actual definition as a new field of research focuses on the use of plasma technology in the treatment of living cells, tissues, and organs.
- This presentation is an review on plasma that is used in medical purposes and also about different types of plasma sources, adapted plasma devices, plasma medicine, applications of physical plasma etc.

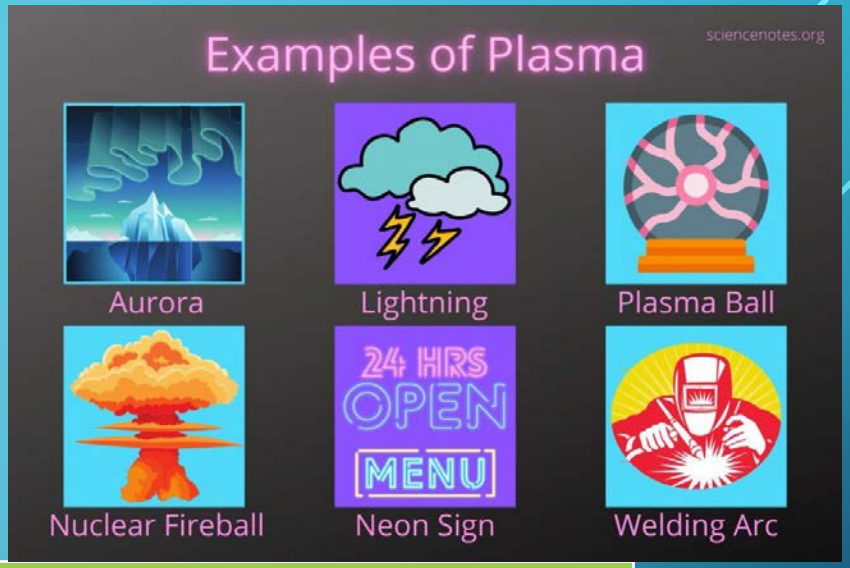
INTRODUCTION

- Plasma is considered the fourth state of matter. The funny thing about that is that as far as we know, plasmas are the most common state of matter in the universe. They are even common here on earth. The biggest chunk of plasma you will see is that dear friend to all of us, the sun.
- The search for new and innovative fields of plasma application for the new century resulted in new approaches solving unmet problems by plasma technology.
- Plasma and medicine are interrelated in modern medical technology, biotechnology and pharmacy.
- Plasma medicine is an emerging field that combines plasma physics, life sciences and clinical medicine.
- Most of the research is in vitro and in animal models. Plasma medicine means the direct application of cold atmospheric plasma (CAP) on or in the human body for therapeutic purposes.

WHAT IS THE PLASMA?

- Non-equilibrium plasmas are weakly ionized gases which contain charged species (electrons, positively and negatively charged ionic species), neutral species (atomic and/or molecular radicals and others), and electric fields. These plasmas also emit radiation that spans wavelengths in the infrared, visible, and ultraviolet (UV) ranges.

EXAMPLES OF PLASMA IN THE NATURE

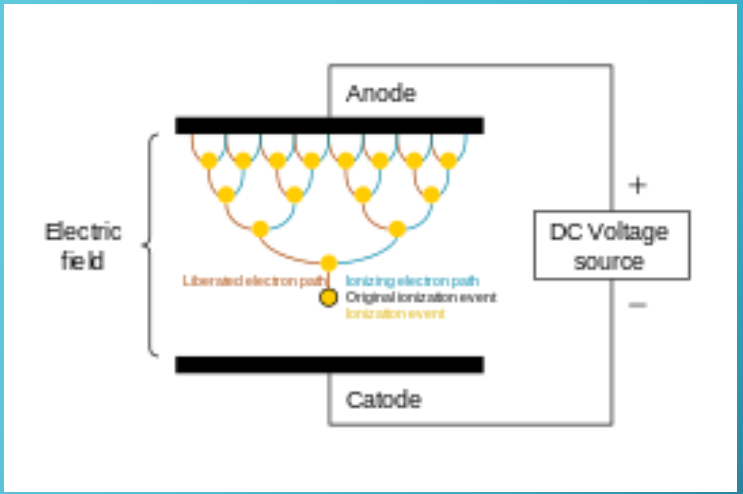


Lightning Aurora Comet tail Solar wind Stars (including the Sun) Interstellar gas clouds Welding arcs Interior of neon signs and fluorescent lights Interior of a plasma ball toy Static electricity Fireball of a nuclear explosion	Earth's ionosphere Earth's magnetosphere Plasma displays of some televisions Rocket exhaust and thrusters Area in front of a heat shield during spacecraft re-entry Interstellar nebula Interstellar and intergalactic medium St. Elmo's fire Fire (if it is hot enough)

ARTIFICIAL PLASMA

- Most artificial plasmas are generated by the application of electric and/or magnetic fields through a gas. Plasma generated in a laboratory setting and for industrial use can be generally categorized by:
- The type of power source used to generate the plasma—DC, AC (typically with radio frequency (RF)) and microwave
- The pressure they operate at: vacuum pressure (< 10 mTorr or 1 Pa), moderate pressure (≈1 Torr or 100 Pa), atmospheric pressure (760 Torr or 100 kPa)
- The degree of ionization within the plasma—fully, partially, or weakly ionized
- The temperature relationships within the plasma—thermal ($T_i = T_e = T_{gas}$), non-thermal or "cold" plasma ($T_e \gg T_i = T_{gas}$)
- The electrode configuration used to generate the plasma
- The magnetization of the particles within the plasma—magnetized (both ion and electrons are trapped in Larmor orbits by the magnetic field), partially magnetized (the electrons but not the ions are trapped by the magnetic field), non-magnetized (the magnetic field is too weak to trap the particles in orbits but may generate Lorentz forces)

PLASMA GENERATION



1- **Electric arc**

With ample current density and ionization, this forms a luminous electric arc (a continuous electric discharge similar to lightning) between the electrodes. Electrical resistance along the continuous electric arc creates heat, which dissociates more gas molecules and ionizes the resulting atoms (where degree of ionization is determined by temperature), and as per the sequence: solid-liquid-gas-plasma, the gas is gradually turned into a thermal plasma.

PLASMA GENERATION

2- **Low-pressure discharges**

- *Glow discharge plasmas*: non-thermal plasmas generated by the application of DC or low frequency RF (<100 kHz) electric field to the gap between two metal electrodes. Probably the most common plasma; this is the type of plasma generated within fluorescent light tubes.
- *Capacitively coupled plasma (CCP)*: similar to glow discharge plasmas, but generated with high frequency RF electric fields, typically 13.56 MHz. These differ from glow discharges in that the sheaths are much less intense. These are widely used in the microfabrication and integrated circuit manufacturing industries for plasma etching and plasma enhanced chemical vapor deposition.
- *Cascaded arc plasma source:* a device to produce low temperature (≈1eV) high density plasmas (HDP).
- *Inductively coupled plasma (ICP)*: similar to a CCP and with similar applications but the electrode consists of a coil wrapped around the chamber where plasma is formed.
- *Wave heated plasma*: similar to CCP and ICP in that it is typically RF (or microwave). Examples include helicon discharge and electron cyclotron resonance (ECR).

PLASMA GENERATION

3- Atmospheric pressure

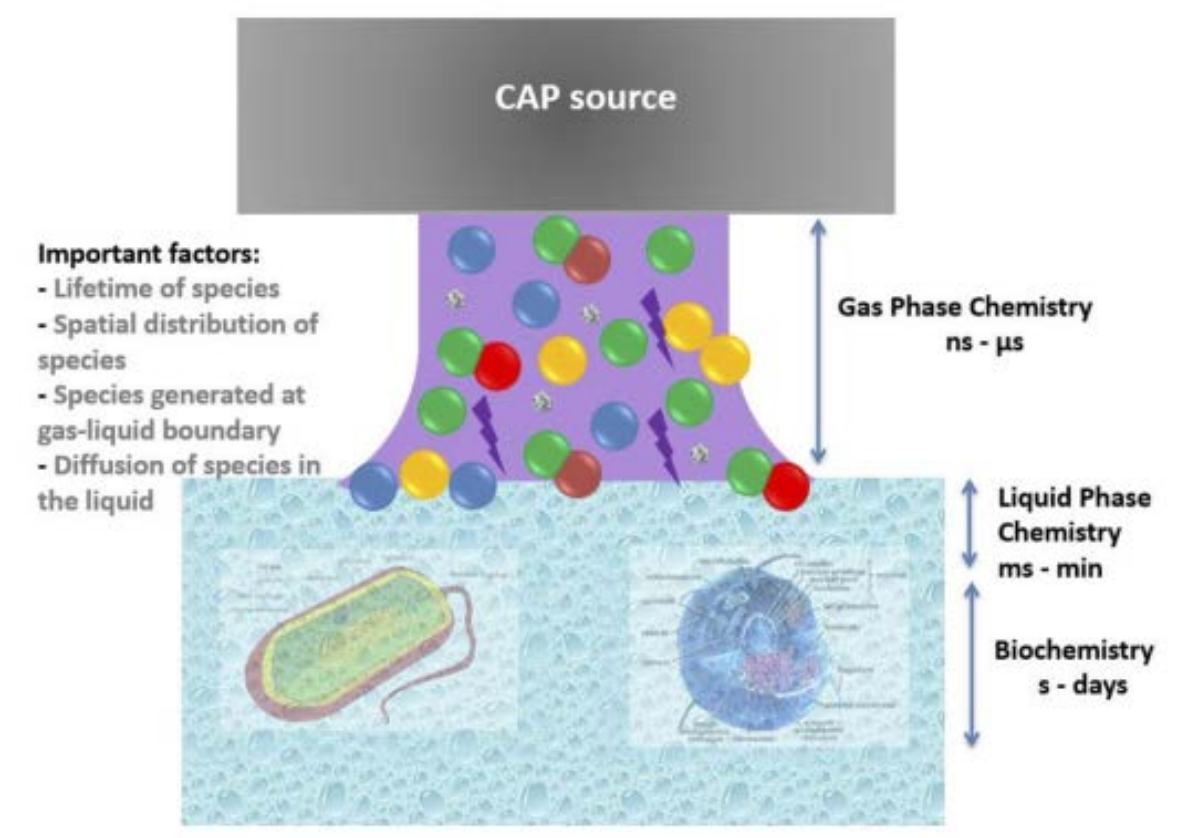
- *Arc discharge:* this is a high power thermal discharge of very high temperature (≈10,000 K). It can be generated using various power supplies. It is commonly used in metallurgical processes. For example, it is used to smelt minerals containing Al_2O_3 to produce aluminum.
- *Corona discharge:* this is a non-thermal discharge generated by the application of high voltage to sharp electrode tips. It is commonly used in ozone generators and particle precipitators.
- *Dielectric barrier discharge (DBD):* this is a non-thermal discharge generated by the application of high voltages across small gaps wherein a non-conducting coating prevents the transition of the plasma discharge into an arc. It is often mislabeled 'Corona' discharge in industry and has similar application to corona discharges. A common usage of this discharge is in a plasma actuator for vehicle drag reduction. It is also widely used in the web treatment of fabrics. The application of the discharge to synthetic fabrics and plastics functionalizes the surface and allows for paints, glues and similar materials to adhere. The dielectric barrier discharge was used in the mid-1990s to show that low temperature atmospheric pressure plasma is effective in inactivating bacterial cells. This work and later experiments using mammalian cells led to the establishment of a new field of research known as plasma medicine. The dielectric barrier discharge configuration was also used in the design of low temperature plasma jets. These plasma jets are produced by fast propagating guided ionization waves known as plasma bullets.
- *Capacitive discharge:* this is a non-thermal plasma generated by the application of RF power (e.g., 13.56 MHz) to one powered electrode, with a grounded electrode held at a small separation distance on the order of 1 cm. Such discharges are commonly stabilized using a noble gas such as helium or argon
- "Piezoelectric direct discharge plasma:" is a non-thermal plasma generated at the high-side of a piezoelectric transformer (PT). This generation variant is particularly suited for high efficient and compact devices where a separate high voltage power supply is not desired.

ATMOSPHERIC-PRESSURE PLASMAS (CAP)

- it was well known that atmospheric-pressure plasma is effective to inactivate microorganisms.
- Therefore, the first and, up to now, main focus of plasma medical applications is in the field of dermatology, especially for skin disinfection/antisepsis, treatment of infectious skin diseases and wound healing.
- Plasma sources for direct or indirect plasma treatment were distinguished, respectively:
- Direct plasma treatment means that biological samples or living tissue serve as one of the electrodes necessary for plasma ignition.
- In indirect plasma treatment, the electrodes are part of the plasma-generating device, only. Thus, there is primarily no electrical contact to the targeted structures.

ATMOSPHERIC-PRESSURE PLASMAS

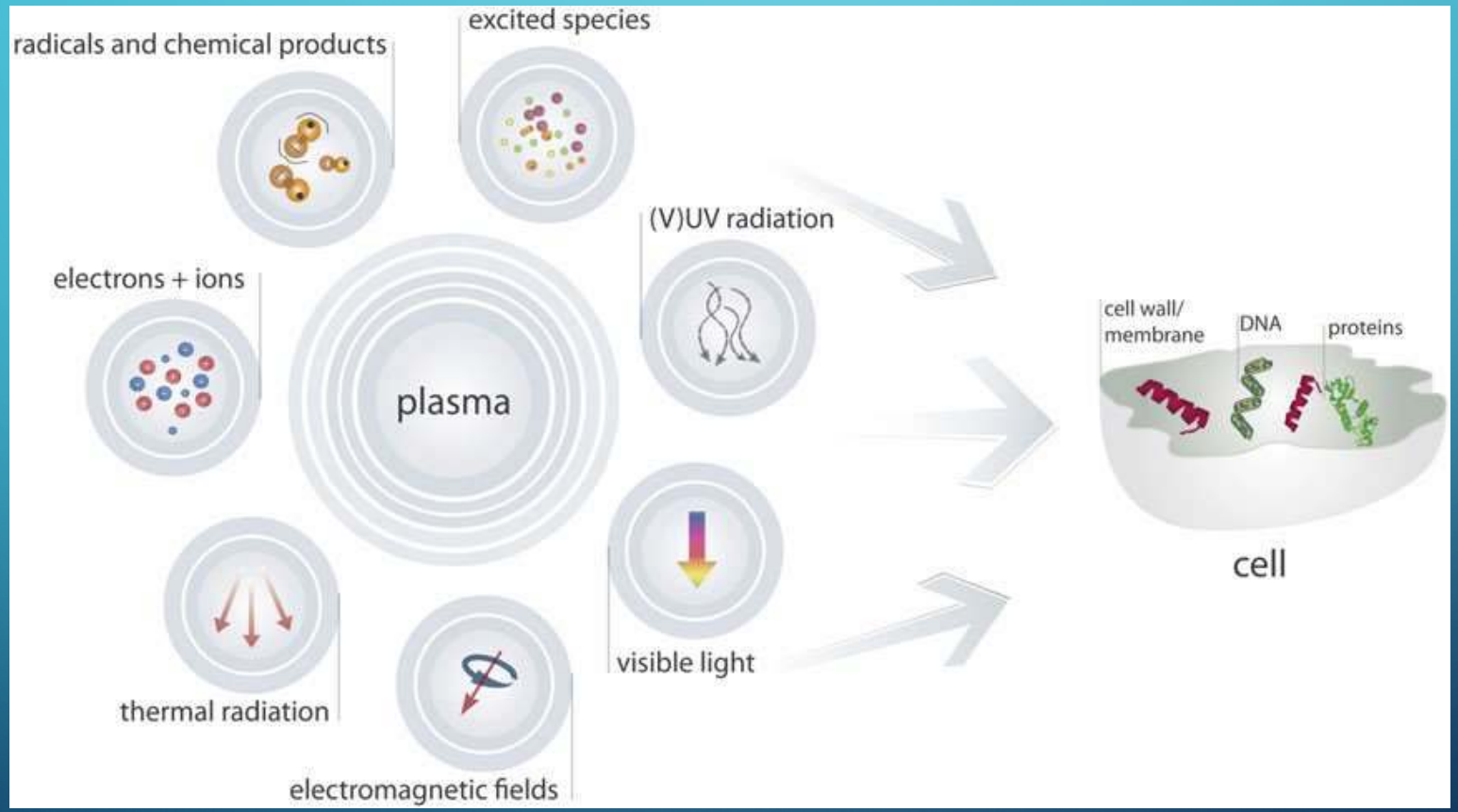
In the plasma afterglow, where air diffusion into the feed gas channel occurs, other secondary reactive species such as O, OH, O3, O2 , NO, and NO2 are generated.



SCIENTIFIC BASIS OF MODERN PLASMA MEDICINE

Studies on the effects of plasma-generated reactive species on biological cells showed that, depending on the operating conditions (power, gas type and flow rate, exposure time, distance between plasma source and target, etc), different outcomes can be achieved. These include cell death (via regulated cell death like necrosis or apoptosis), cell detachment, change in cellular morphology and heterotrophic pathways, change in cell motility, cell proliferation, etc.

SCIENTIFIC BASIS OF MODERN PLASMA MEDICINE



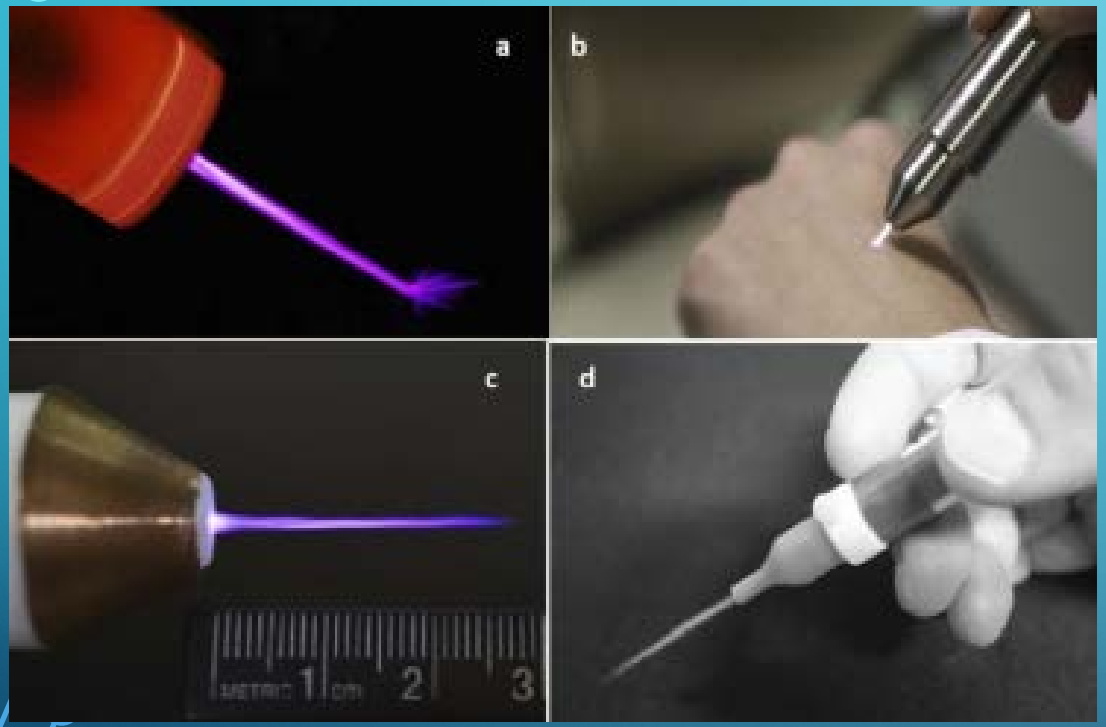
ATMOSPHERIC-PRESSURE PLASMAS FOR MEDICAL PURPOSES

Investigations into the interaction of CAP with biological cells and tissues showed that the effects of LTP are mediated primarily by chemically reactive oxygen species (ROS) and reactive nitrogen species (RNS). The biological effects of many of the above-listed reactive species are well known in cell biology. These species can interact with cell membranes, enter the cells and increase the intracellular ROS concentrations, which may lead to DNA strand breaks, mitochondria damage, and may compromise the integrity of other organelles and macromolecules (such as proteins).

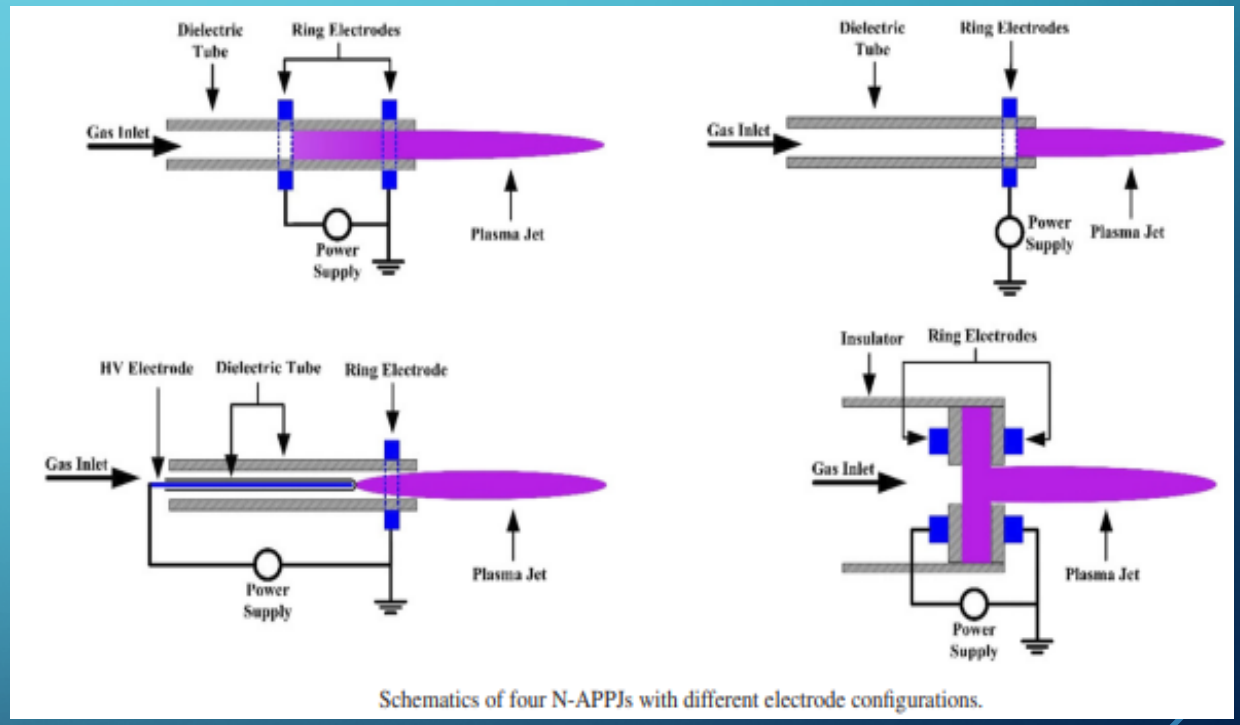
ATMOSPHERIC-PRESSURE PLASMAS FOR MEDICAL PURPOSES

For example, the hydroxyl radical (OH) causes peroxidation of unsaturated fatty acids which are a major component of the lipids constituting the cell membrane. Another byproduct of plasma application is hydrogen peroxide (H2O2), which possesses strong oxidative properties that affect, via the peroxide ions, lipids, proteins, and DNA. Nitric oxide (NO) is another species that can be generated by plasma as it interacts with air. The NO molecule is known to have several biological effects, which include the regulation of immune-deficiencies, induction of phagocytosis, proliferation of keratinocytes, and regulation of collagen synthesis.

ATMOSPHERIC-PRESSURE PLASMAS FOR MEDICAL PURPOSES

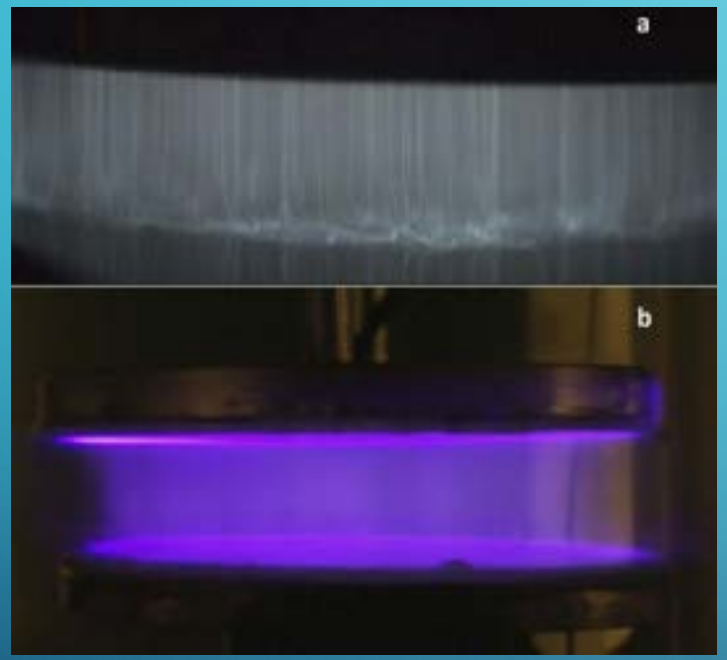


Photographs of N-APPJs. (a) The plasma pencil driven by pulsed DC (image by ODU/M. Laroussi); (b) the kINPen driven by RF power (image by INP/Th. von Woedtke); (c) Plasma jet driven by DC. Reproduced with permission from A. Shashurin; (d) Plasma jet driven by a piezoelectric transformer. Reproduced with permission from C. Tendero

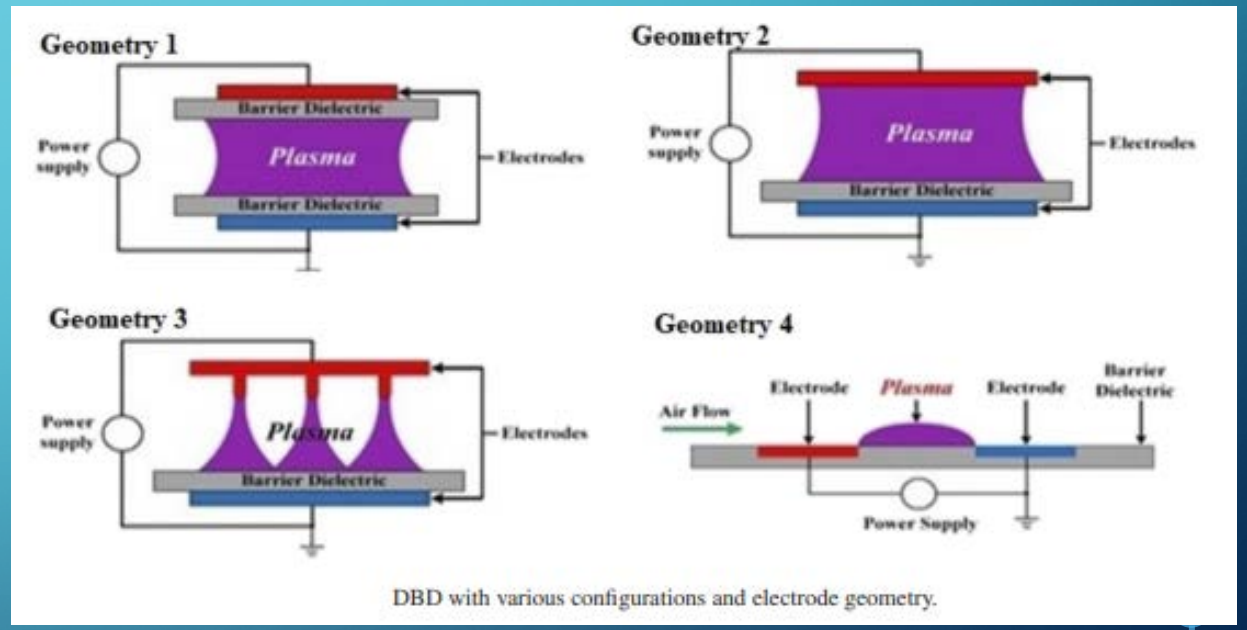


Schematics of four N-APPJs with different electrode configurations.

ATMOSPHERIC-PRESSURE PLASMAS FOR MEDICAL PURPOSES

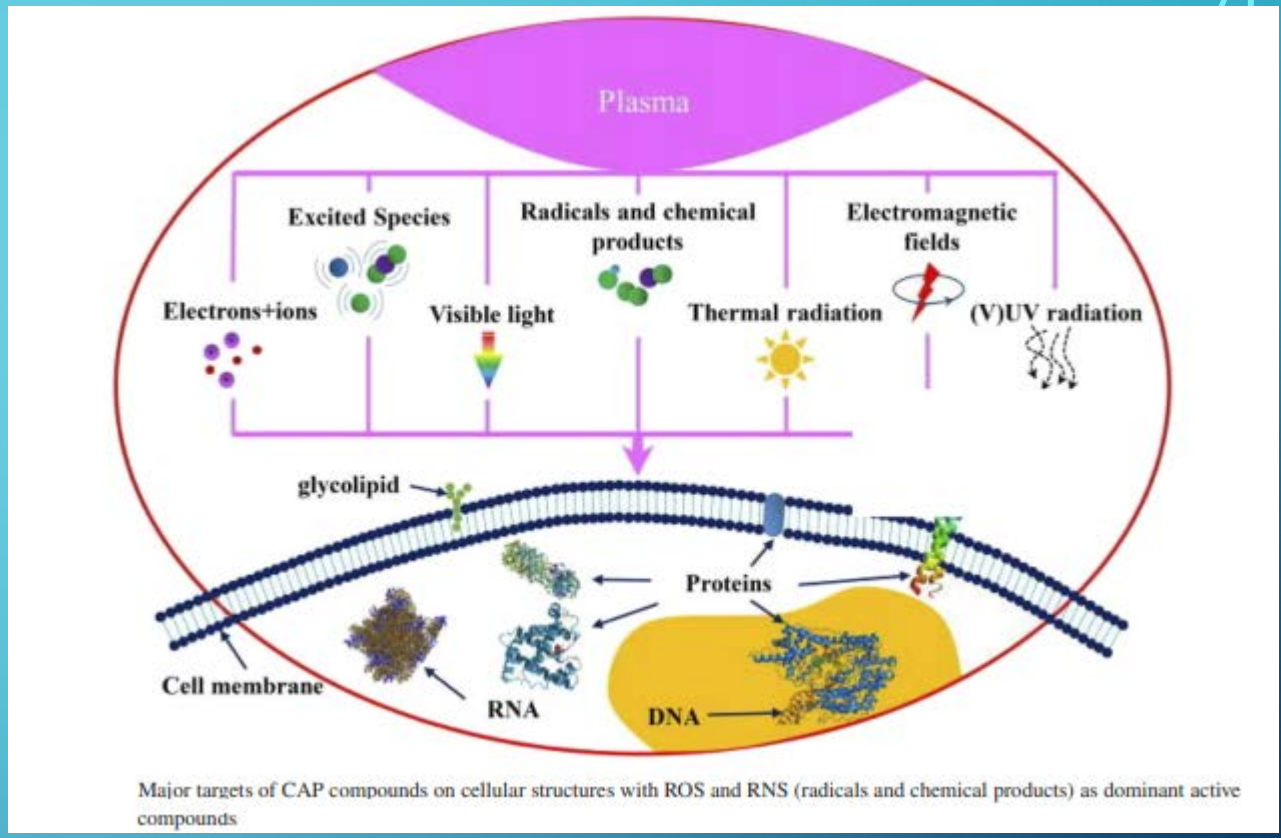


Images of a DBD-generated filamentary plasma (a) and that of diffuse DBD plasma (b). Images (a) and (b) are by Mounir Laroussi.



DBD with various configurations and electrode geometry.

CAP FOR MEDICAL PURPOSES



Major targets of CAP compounds on cellular structures with ROS and RNS (radicals and chemical products) as dominant active compounds

Plasma influences

- **specific detachment of cells from matrices as well as from cell clusters**
- **cell migration**
- **cell proliferation and angiogenesis**
- **expression of cell surface proteins/cell adhesion molecules (integrins, cadherins,)**
- **DNA integrity**
- **apoptosis induction, inactivation of cancer cells**
- **reversible cell membrane permeabilization (“plasma poration”)**
- **blood coagulation by direct influence on the coagulation cascade.**

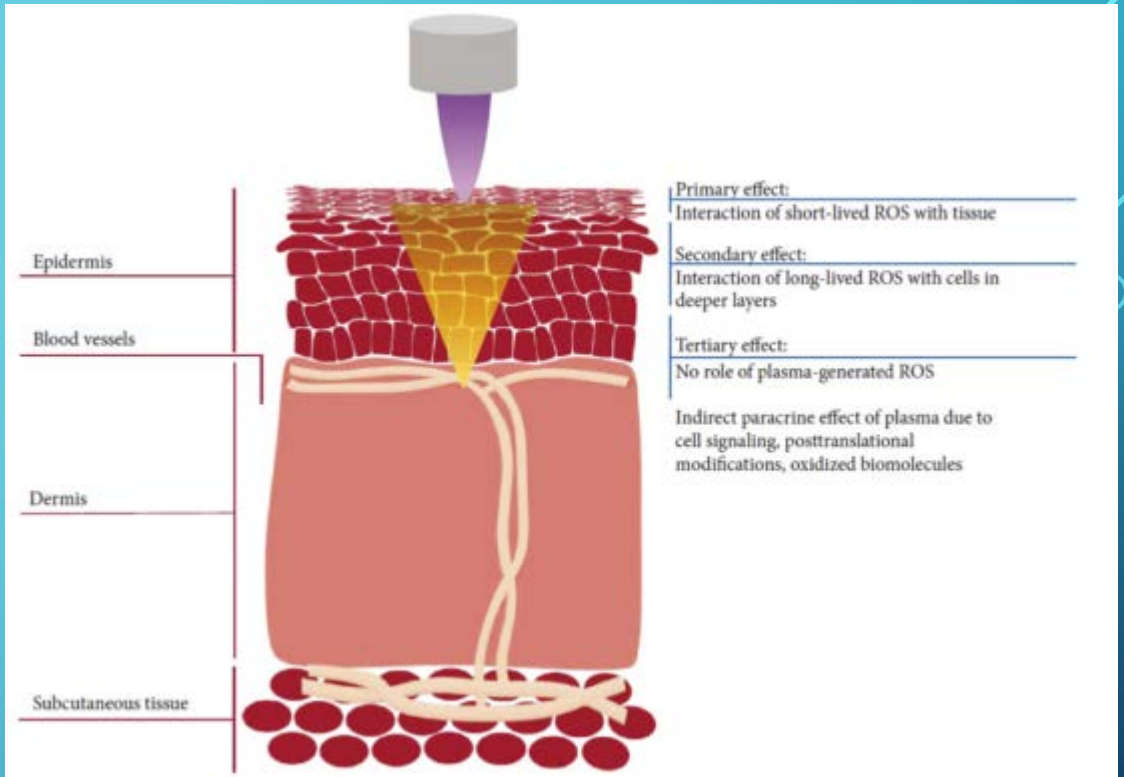
CAP FOR MEDICAL PURPOSES

- Generally, there are three mechanisms under discussion which may explain plasma effects in deeper skin and tissue layers:

I. Diffusion of plasma-originated or secondary generated long-lived ROS and RNS into deeper tissue layers;

II. Action of proteins with plasma-caused posttranslational modifications or other oxidized biomolecules;

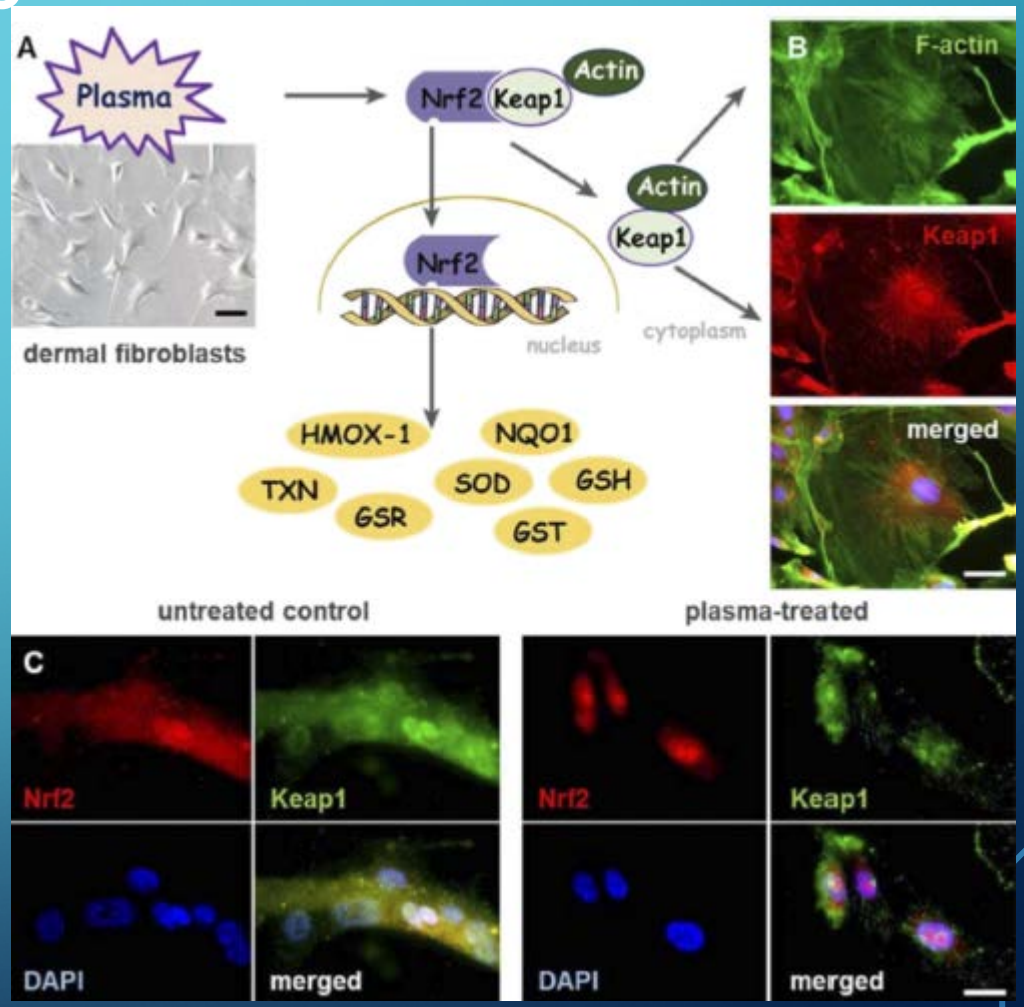
III. Stimulation of cell–cell communication after direct exposure of surface cells to plasma or plasma-generated species, respectively



Discussed mechanisms of plasma-caused tissue effects;

CAP FOR MEDICAL PURPOSES

Cold physical plasma triggers nuclear translocation of Nrf2, and induces colocalization of Keap1 with actin filaments in the cytoplasm. (A) Dermal fibroblasts (bright field image, left) were isolated from SKH1 mouse skin and exposed to cold physical plasma-derived ROS/RNS. Upon nuclear translocation of the nuclear factor erythroid 2-related factor 2 (Nrf2), plasma significantly altered antioxidant and phase II detoxification enzymes and proteins (e.g., heme oxygenase 1 (HMOX-1), NADPH quinone oxidoreductase 1 (NQO1), thioredoxin (TXN), glutathione reductase (GSR), superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione (GSH) etc). (B) Cytoplasmic localization of Kelch-like ECH-associated protein 1 (Keap1) was detected immunohistochemically by anti-Keap1 antibody (red). Colocalization of Keap1 with actin filaments was visualized by staining with fluorescein isothiocyanate (FITC)-phalloidin (green). (C) Subcellular localization of Keap1 (green) and trans-localization of Nrf2 (red) from the cytoplasm to the nucleus were detected immunohistochemically by anti-Keap1 and anti-Nrf2 antibodies in plasma-treated (right panel), but not control fibroblasts (left panel). Scale bars 100 μm (A), 50 μm (B) and (C). Reproduced from

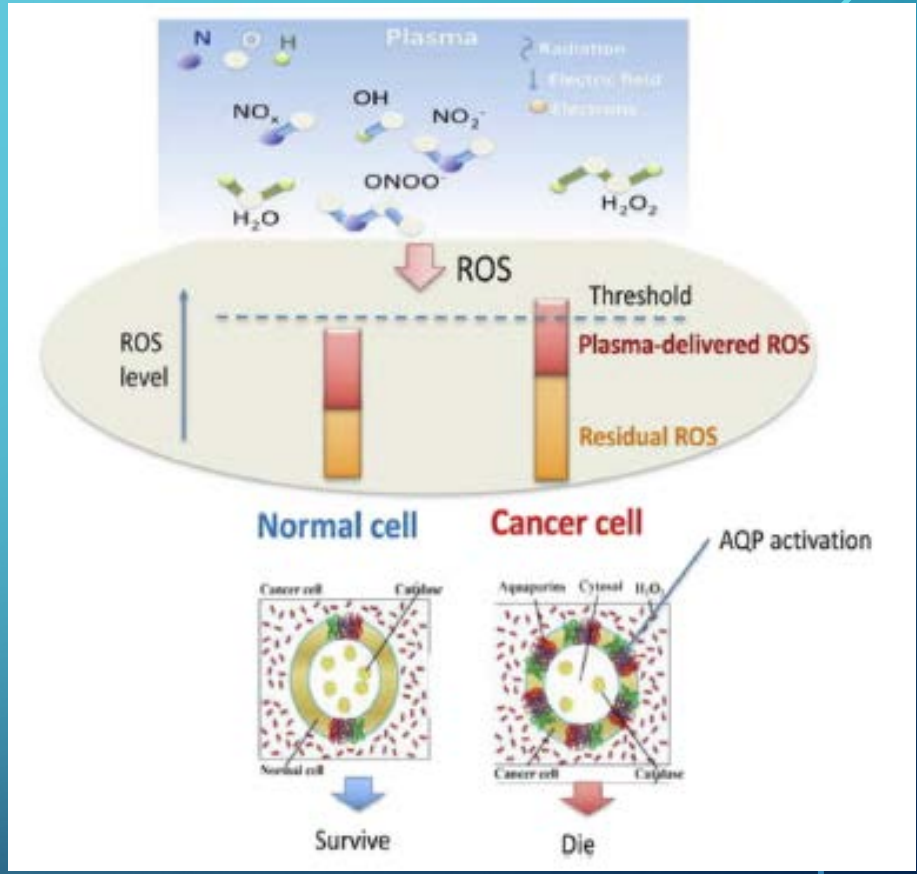


CAP ON THE CANCER CELLS

The enhanced plasma sensitivity of cancer cells is explained by a selective stronger rise of intracellular ROS/RNS caused by specific characteristics of cancer cells. The first barrier that is tackled and has to be overcome by plasma-caused ROS/RNS is the cell membrane. Because of its hydrophilicity, the direct transmembrane diffusion of ROS/RNS is limited. Transmembrane channel proteins called aquaporins serve as water channels facilitating the transport of water between cells but also enabling diffusion of small hydrophilic molecules.

CAP ON THE CANCER CELLS

An increased expression of aquaporins in cancer cells may be one cause for increased impact of ROS/RNS and resulting in the higher sensitivity of cancer cells to plasma treatment. On the other hand, the diffusion of ROS/RNS through phospholipid membranes depends on the cholesterol content providing membrane stability and fluidity. The amount of cholesterol is often reduced in cancer cells. Lipid peroxidation caused by ROS/RNS may contribute to additional pore generation in such cholesterol-depleted cell membranes. Selected aspects of plasma selectivity to cancer cells in comparison to normal cells are presented schematically.



INACTIVATION OF MICROORGANISMS BY CAP

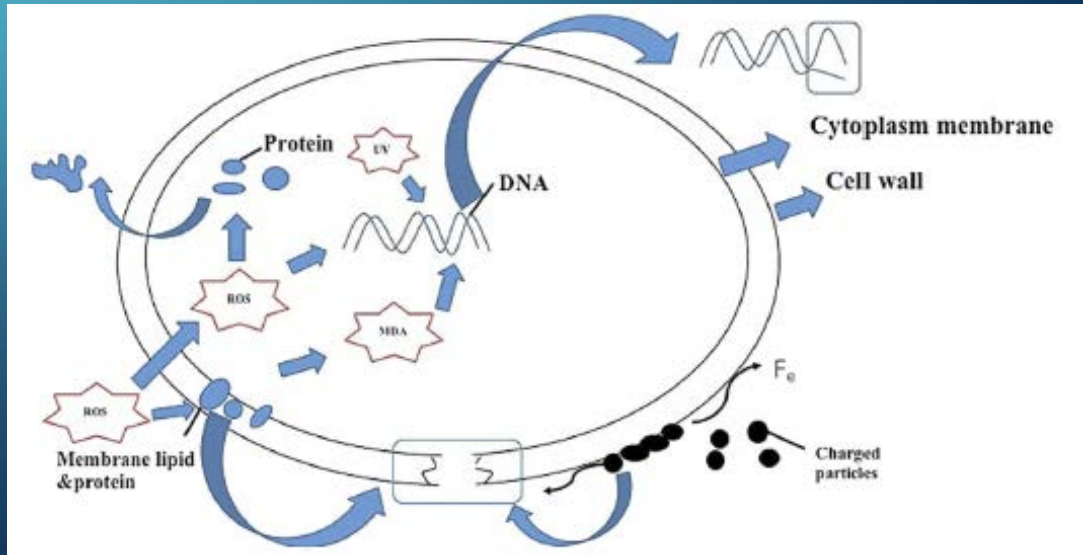
Similarly to mammalian cells including cancer cells, microbicidal CAP effects are mainly attributed to the activity of ROS and RNS. UV effects as in low-pressure plasma do not seem to be the major inactivating factor because of low UV doses and UV absorption by ambient atmospheric air. Some proposed and mainly ROS/RNS-based inactivation mechanisms of microorganisms are depicted schematically.

Antimicrobial CAP effects are mainly attributed to three general actions:

(i) Permeabilization or damage of cell membrane or wall

(ii) Modification or damage of intracellular proteins

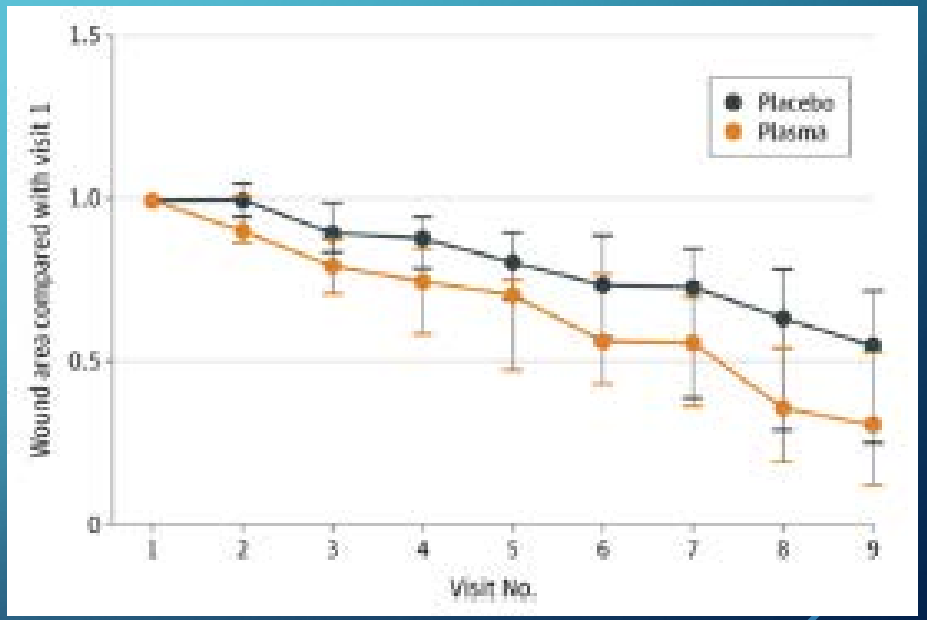
(iii) DNA damage.



THERAPEUTIC APPLICATIONS OF CAP

1. Wound Healing:

Wound size reduction in 62 diabetic foot ulcers (placebo:31, plasma: 31) after eight CAP treatments within 14 days (daily treatment for five consecutive days followed by three treatments every second day) in relation to start of therapy; visit nine: two to three days after the last treatment.



THERAPEUTIC APPLICATIONS OF CAP

2. *Dermatology*

Mainly based on the antimicrobial and antiviral effectivity of CAP, single clinical investigations have demonstrated the successful CAP effect on onychomycosis (fungal infection of the nail) and warts.

The repeatedly reported CAP effect to enhance skin permeability and to enhance the transcutaneous penetration of substances may be promising both for cosmetic purposes and for transcutaneous drug release, but needs much more research!

THERAPEUTIC APPLICATIONS OF CAP

3- *Cancer treatment*

In the treatment of actinic keratosis, a precancerous skin disease which can develop if left untreated, in skin cancer called squamous cell carcinoma, CAP achieved good results in the first patient studies.

The state of knowledge on CAP effects on cancer cells, including immunostimulatory effects, together with the first clinical applications, characterizes cancer treatment as the next challenging but promising field of clinical CAP application. Further efforts have to be made to find out if CAP will be able to mark a ‘paradigm shift in cancer therapy’ as was predicted in 2011.

THERAPEUTIC APPLICATIONS OF CAP

4- Dentistry

Another field of long-term research on CAP application is oral medicine and dentistry. Here, several fields of application are thinkable, ranging from implantology, treatment of perimplantitis, treatment of teeth and tooth root channels, treatment of oral wounds and infections up to rather aesthetic applications such as tooth whitening.

THERAPEUTIC APPLICATIONS OF CAP

Other applications

- Finally, an intensively researched field hat is not a direct CAP application in medicine is the use of plasma-treated liquids, frequently called PTS, plasma-conditioned liquids, PAL, plasma-activated water (PAW), or plasma-activated medium (PAM). Liquid phases do not only serve as a transient transport medium to bring ROS/RNS from the plasma/gas phase to the biological target (see section 4.2), but plasma treatment of liquids also results in the enrichment of new chemical species and subsequent changes of their biological characteristics, making them useful for several applications.

THERAPEUTIC APPLICATIONS OF CAP

Most research with respect to medical application of plasma treated liquids has been done in cancer treatment. Here, two application strategies are followed:

(i) direct injection into bulk tumors,

(ii) injection/infusion into the peritoneal cavity in the case of metastasized intraperitoneal tumors, so called carcinomatosis.

THANK YOU