PLASMAS FROIDS
POUR LE BIOMEDICAL

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PLASMA MEDECINE

Biomedical Applications
- Surface Modification
- Therapeutic Applications
- Biological Decontamination

Plasma Sources
- Atmospheric Pressure Plasma Sources
  - Favoured
  - Required
  - Favoured/Required

Plasma Physicists, Engineers, Biologists, Biochemists, and Medical doctors

H.-R. Metelmann et al. (eds.), Comprehensive Clinical Plasma Medicine, Springer Nature 2018
Medical applications of low temperature non-thermal plasmas

A new (old) field...

Violet rays: since the beginning of the 20th century

In use for decades, but its efficacy has not been evaluated!
Et maintenant en français…

Pour un Traitement
Electro Thérapeutique
efficace

Exigez la Marque :

HOLO-ELECTRON

LA SANTÉ POUR TOUS!

Et demandez
NOTRE PRIX-COURANT

Édité par "HOLO-ELECTRON" pas d'autres remèdes et équivalents courants

Les rhumatismes guéris par
l'Electricité

Douleurs et rhumatismes disparaissent comme par enchantement sous l'action vivifiante de l'appareil

SALVALUX

Branché sur une prise du courant, Salvalux était des radiations combinant les effets bienfaisants et reconnus de la chaleur, de la lumière et de l'électricité. Des milliers de malades (références contrôlées) doivent la fin de leurs souffrances à ces rayons dits "rayons violets".

DIX JOURS A L'ESSAI GRATUITEMENT

Demandez dès aujourd'hui le bon d'essai gratuit du "SALVALUX" et notre livre N° 19 de 50 pages et 125 gravures, le tout sans frais ni engagement pour vous. Si vous n'êtes pas satisfait des résultats, dans les dix jours, vous nous le retournez simplement.

Etablissements SALVALUX
25, Boulevard Bonne-Nouvelle - PARIS-2e
Already in use but **not** cold plasmas

**Electro-surgery**  
**Electro-cauterization**

**Superficial resection of diseased tissue**

**Professor Horace Roman,**  
MD, PhD,  
CHU University Hospital,  
France.

Rouen
**PLASMA MEDECINE**

**Timeline:**
- **1996**: Decontamination of surfaces and media with atmospheric pressure plasma (USA).
- **1997**: Plasma Dynamic Therapy of wounds, fibroblasts proliferation (Russia).
- **1998**: Disinfection for wound healing (USA – AFOSR).
- **1998**: Detachment, apoptosis (Netherlands).
- **2002**: FDA approval of plasma jet in dermatology (USA).
- **2006**: Cancer applications (North America, Europe, Asia).
- **2008**: Clinical trial for wound healing (Germany).
- **2010**: Class IIa medical device certification (Germany).
- **2013**: Plasma Activated Media for Cancer (Japan).

**First Decade: Early Foundation of Plasma Medicine**

**Second Decade: Rapid Growth of Plasma Medicine**

*Plasma* 2018, 1, 5; doi:10.3390/plasma1010005
PLASMAS FOR THERAPEUTIC APPLICATIONS

Medical applications of low temperature non-thermal plasmas

Air environment at atmospheric pressure
T_{gas} < 40°C, cooler even better
Liquid interface

Therapeutic applications require:

1/ non-equilibrium plasmas:
   Te = 1-10 eV ≠ Tg = 300 K (0.025 eV)

2/ atmospheric pressure

1+2 = real challenge → what kind of electrical discharges?
Glow discharges

Atmospheric pressure
Pd=100 → d=1.3 mm!

1: primary electron
2: avalanche in a few ns
3: streamer velocity: $10^8$ cm/s
4: conductive channel diameter: 100s µm
5: glow to arc transition → thermal plasma

Yu. Raizer, Gas Discharge Physics

How to avoid the glow to arc transition?
NON-EQUILIBRIUM PLASMAS AT ATMOSPHERIC PRESSURE

Key point: how to avoid the glow to arc transition?

1/ external preionization:

- Overlapping the streamer heads

  Very efficient but complex and expensive
  High value products: high power lasers

2/ current limitation:

- résistive

  Gap 4.5 cm, He/air

- Capacitive (DBD)
  Gap 25 cm, Ne/SF₆/F₂

Classical discharges = discharges between 2 electrodes inside a closed chamber
OPEN DISCHARGES

**FE-DBD**

A. Fridman Drexel University

Treatment over a large surface area but short gap (few mm): external treatment

**Direct DBD**

- Tissue exposed to plasma
- Charged, excited species, radicals
- UV, electric field
- Tolerable current through tissue

**Surface DBD**

- Plasma afterglow
- No current, very small amount of ions
- Mainly long-lived reactive species
- UV rays
treatment over a large surface area but short gap (few mm): external treatment

RF discharge (usually high Tg)
Needle tip is at room temperature: biomedical applications allowed

"a non-destructive atmospheric plasma source for fine surface treatment of (bio)materials."

Room-temperature atmospheric pressure plasma plume for biomedical applications
M. Laroussi\textsuperscript{a)} and X. Lu \textit{APPLIED PHYSICS LETTERS} \textbf{87}, 113902 (2005)

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{plasma_jets}
\caption{(Color online) Photograph of the plasma plume in contact with human skin.}
\end{figure}

Dynamics of an atmospheric pressure plasma plume generated by submicrosecond voltage pulses
XinPei Lu and Mounir Laroussi\textsuperscript{a)} \textit{JOURNAL OF APPLIED PHYSICS} \textbf{100}, 063302 (2006)

\textbf{plasma pencil,} is driven by few hundred nanosecond wide pulses at repetition rates of a few kilohertz. Correlation between current-voltage characteristics and fast photography shows that the \textbf{plasma plume is in fact a small bulletlike volume of plasma traveling at unusually high velocities.}
PLASMA JETS

DC microplasma

RF plasma jet

microwave

HV-LF excitation

Diameter: 4 mm, Length: 50 cm

in air

(B)

Plasma plume

Petri dish

ϕ=15 μm
PLASMA JETS

All kinds of electrical excitations: DC, AC, RF, MW, continuous or pulsed

Rare gases (with or without admixtures: O₂, N₂, H₂O₂,…) but also pure N₂ or Air

Unlimited terminology: APPJ, Plasma Plume, Plasma Pencil, Plasma Gun, Plasma Torch,…

Discharge operated in a non-sealed electrode arrangement
plasma « expansion » outside the discharge region
either through high gas flow or determined by the electric field

Plasma or afterglow (effluent) delivery on targets
Coaxial DBD

Applied voltage (pulsed): 3–40 kV (100ns–10µs)
(typically 5 kV, 500 ns)

Frequency: 1–50 kHz (typically 20 kHz)

Gas: He, Ar, with or without O₂/N₂/H₂O

Gas flow: 50 to 5000 sccm
The plasma jet is not continuous; it is rather a streamer guided by the gas channel.

The velocity of the “guided streamer” is of several hundreds km/s.

Stable at atmospheric pressure
Low gas temperature $\approx 300-350$ K
Possible use for endoscopic treatments
Plasma Gun
Splitting
Splitting
Transfer

Mixing
Visualization of KinPen plasma jet source with shielding gas. (a) photograph and (b) schematic and visualization of the shielding gas curtain by CFD simulation.

Photograph (a) and sketch (b) of the modified μAPPJ (so-called X-jet) setup.

Free jet in ambient air

Pulse repetition rate influence

Upstream and downstream shift of the laminar to turbulent transition

1000 Hz
500 Hz
250 Hz

Grounded target
Floating potential target

Plasma OFF

FLUID DYNAMICS
Plasma jets generate Electric Fields

Electric field along the capillary

Ionization front Plasma tail

Electric field in the plasma plume

Electric field under 3 mm liver layer
kINPen™: basic module

- Atmospheric pressure plasma
- Cold plasma jet
- Variable in length (some mm)
- Easy to use and handle
- Generation of UV/VUV radiation and chemically active species (radicals)
- CE certified

INP Greifswald

Dimension:
L=190mm, Ø 20mm

Weight:
170g

HF-Voltage:
1.1MHz; 2…6kV

Gas temperature:
30°C - 150°C

Gas flow:
1-5 slm

kINPen 09 (@ INP Greifswald)
Phase II study: MicroPlaSter

distance to wound controlled by ultrasounds
The new device - MicroPlaSter β

- Used gas: argon
- Voltage = 50 - 100 V
- Frequency = 2.3 GHz
- Power = 100 W

Microwave Plasma
DBD based medical device
CELLULAR EFFECTS

Cellular effects *in vitro* induced by cold atmospheric plasmas

**Lethal effects:**
- Inactivation/killing of microorganisms (prokaryotic cells) including antibiotic-resistant pathogens
- Inactivation or killing of mammalian cells (eukaryotic cells) including cancer cells mainly via induction of apoptosis depending on intensity (time) of plasma impact

**Non-lethal effects:**
- Influence on/stimulation of metabolism of microorganisms (prokaryotic cells)
- Specific/selective effects on mammalian cells (eukaryotic cells):
  - Influence on cell migration
  - Influence on expression of surface proteins responsible for cell-cell and cell-matrix interactions
  - Influence on/stimulation of cell proliferation
  - Influence on/stimulation of angiogenesis
  - Reversible impact on DNA integrity, influence on cell cycle
  - Reversible permeabilization of cell membranes (“plasma poration”)
  - Non-thermal blood coagulation
Mechanisms of the biological effects of cold atmospheric plasmas in vitro:

- Significant biological plasma effects are caused by plasma-induced changes to the liquid environment of cells
- Reactive oxygen and nitrogen species (ROS, RNS/RONS) generated in or transferred into liquid phases play a dominant role in biological plasma effects.

### Reactive oxygen species (ROS) vs. Reactive nitrogen species (RNS/RONS)

<table>
<thead>
<tr>
<th>Reactive oxygen species (ROS)</th>
<th>Reactive nitrogen species (RNS/RONS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide: $\text{O}_2^-$•</td>
<td>Nitric oxide: •NO</td>
</tr>
<tr>
<td>Hydrogen peroxide: $\text{H}_2\text{O}_2$</td>
<td>Nitrogen dioxide: •NO$_2$</td>
</tr>
<tr>
<td>Hydroxyl radical: •OH</td>
<td>Peroxynitrite: ONOO$^-$</td>
</tr>
<tr>
<td>Singlet oxygen: $^1\text{O}_2$</td>
<td></td>
</tr>
<tr>
<td>Ozone: $\text{O}_3$</td>
<td></td>
</tr>
<tr>
<td>Organic radicals: RO•, RO$_2$•</td>
<td></td>
</tr>
</tbody>
</table>
THERAPEUTIC APPLICATIONS

- Sterilization and decontamination
- Skin and tissue sterilization
- Hygiene

- Dermatology
- Dental care
- Blood coagulation and primary hemostasis
- Inflammation

- Wound and ulcer care (clinical studies)
- Antitumoral effect and tumor treatment (case studies)

Treatments *in vitro* and *in vivo* (animal models and clinical tests)
CHRONIC WOUNDS HEALING

– Open, highly infected skin lesion
– Persistent for more than 3 months without healing progress
– Not cured after 12 months of therapy
– Main causes are circulatory disorders of veins or arteries
– Diabetes, spinal cord injury, other disorders that cause immobility
– Risk factors: age, pregnancy, obesity, smoking, former severe leg injury, venous thrombosis, standing and sitting for long periods
– Standard wound care: debridement, saline solution, modern wound dressing, compression stocking
MicroPlaSter
Pat.72: Therapy area
CHRONIC WOUNDS HEALING

– UV radiation and reactive gas species (i.e. O₃)
  Disinfection

– Nitric oxide (NO) or other nitrogen species (NOₓ)
  Stimulation of tissue regeneration
  Wound acidification

– Electric current
  Stimulation of micro-circulation and angiogenesis

The results of basic research gave rise to the early hypothesis that plasma-supported wound healing may be based not only on the reduction of bacterial colonization or elimination of wound infection, but also by direct stimulation of regeneration of damaged tissue. Based on this hypothesis, the concept of plasma-supported wound healing was developed. This is based on a combination of cleaning and antisepsis on the wound surface, with a stimulation of tissue regeneration in deeper wound areas (Fig. 1.4) [44–46].

Impeded wound healing is a big challenge for both patients and clinics. Despite different etiologies, this kind of wound is frequently characterized by disturbed synchronicity of the different processes of wound healing, wound infections, accumulation of liquid, necrosis of the wound edge, excessive tissue neogenesis, and increased release of proteinases and cytokines [47]. Proteinases disturb the generation of the extracellular matrix and inhibit the migration of fibroblasts and keratinocytes that is essential for successful re-epithelialization in later phases of wound healing. Besides a resulting deceleration and inhibition of wound closure, an increased number of immune cells is detected [48]. Usually, immune cells are attracted from the blood and the surrounding tissue fluid to the wound, which prolongs the inflammatory phase of wound healing. Persistence of immune cells is also given in low-germ chronic wounds. It is not yet completely clear whether this is the cause or the result of disturbance to wound healing. There are several signs that a misguided cellular redox signaling will hold the balance of wound inflammation. The concept of redox balance describes homeostasis of the oxidative and reductive processes in cells and tissues. Reactive oxygen and nitrogen species (ROS, RNS/RONS) or radicals, which are normally generated by the respiratory chain or in conjunction with inflammations, are eliminated by enzymatic and non-enzymatic defense mechanisms [49]. It was supposed for a long time that such reactive species alone cause cell-damaging effects. Nowadays, their necessity for cellular processes beyond pathophysiological effects is well known [50, 51].

H.-R. Metelmann et al. (eds.), Comprehensive Clinical Plasma Medicine, Springer Nature 2018
CANCER TREATMENT

Cold atmospheric plasmas are able to induce apoptosis in cancer cells

*in vitro*
  Drexel Plasma lab (Fridman et al) *(1st 2007)*

*in vivo*
  PLASMED – GREMI, CIPA-TAAM, CBM, Germitech, INEL, CERB *(1st 2009)*

Antitumor activity of plasma has been demonstrated *in vitro* on:
  Melanoma (G361, B16, A2058)
  Glioblastoma (U87MG)
  Hepatocellular carcinoma (BEL-7402, HepG2)
  Colorectal carcinoma (SW480, HCT-116, COLO320DM)
  Lung carcinoma (A549, H460)
  Breast carcinoma (MCF-7)
  Cervix carcinoma (HeLa)
  Oral carcinoma (HSC-2, SCC-15)
  Pancreatic carcinoma (MiaPaca, COLO357)
  …

non systemic treatment with little or no side effects
CANCER TREATMENT

*in vivo* antitumoral activity
HCT-116, colon

→ increase of mice lifespan of 115%
CANCER TREATMENT

HCT 116  Colon

NTP  FE DBD treatment

→ anti-metastatic effect
Antitumor action of Plasma Gun and Chemo on Pancreas

L. Brullé et al PLOS ONE, 7, DOI: 10.1371/journal.pone.0052653 (2012)

4 mouse groups:
Ctrl, Chemo, Plasma and (Chemo+Plasma)

Plasma gun delivery on externalized pancreas
(10 min, 2 kHz, 3 fractions)

Gemcitabine
200 mg/kg each 5 days

Plasma and Chemo treatment

Beneficial effect of combined treatment

Effects on tumor volume

Effect on tumor weight the day of euthanasia (D36)
Fig. 3. (a) Infected cancer ulcer of the tongue in an area with pathohistologically confirmed cancer cells and (b) wound healing under CAP treatment.  

*Clinical Plasma Medicine*

http://dx.doi.org/10.1016/j.cpme.2015.02.001
MECHANISMS

– RONS generally acknowledge to be important in plasma therapeutics
– E-fields and photons are important in some cases (e.g. gene transfection/transdermal delivery; photon-induced chemistry)

Existing therapies using RONS:
– antibiotics
– antifungals
– antiparasiticals
– cancer therapy wide recognition of positive role of RONS in cancer therapy
  - PDT ($O_2(a)$)
  - radiation
  - chemo

Plasma-generated RONS effects are confined to near-surface regions and are applied on timescales short compared to biological responses

BUT
Observed plasma therapeutic effects suggest longer time and length scales are involved in plasma therapeutics
MECHANISMS

Hypothesis: plasma triggers a therapeutic response via RONS

1. Burst (10-100s) of RONS from plasma react with liquid and then layer of surface cells
2. Generation of longer-lived species: H₂O₂, oxidized/nitrated proteins, peptides, amino acids, lipids, etc.
3. These species diffuse to and enter cells or act as ligands to membrane surface receptors
4. This initiates cell responses: DNA damage, cell cycle arrest, and other redox mediated stresses associated with mitochondria
5. Cells try to adapt, e.g. by generating anti-oxidant enzymes
6. Cells too weak or unable to adapt may die, strengthening the organism
7. Stressed cells will communicate to adjacent and distant cells, e.g. via release of cytokines
8. Immune system stimulations and/or blood flow or oxygenation may results
9. Net result is similar to what is intended by immune system response: trigger and activate tissue repair, protect against infections, destroy tumours

David Graves, UC Berkeley
How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis)

Michael Ristow\textsuperscript{a,b,*}, Kim Zarse\textsuperscript{a}

Plasma-generated RONS both \textit{simulate} and \textit{stimulate} natural healing responses
Energy transport through multiple interfaces

Core plasma power input ➔ active plasma chemistry
Core plasma & effluent interface ➔ steep gradients
Effluent region ➔ passive plasma chemistry
Effluent & liquid interface ➔ multi-phase interaction
Liquid solution ➔ liquid chemistry
Liquid & bio interface ➔ bio-chemistry
Challenges & Opportunities

- **Multiphase interfaces:**
  - Plasma – gas – liquid – surface (solid)

- **Multispecies:**
  - Electrons, pos. ions, neg. ions, neutrals, radicals, excited species, photons

- **Multiscale problem – time:**
  - Electron dynamics: ps – ns
  - Ion dynamics: 100 ns – µs
  - Plasma chemistry: 100 µs – ms
  - Surface chemistry: s – min

- **Multiscale problem – space:**
  - Surface structures: nm – µm
  - Charged particle gradients: µm – m
  - Neutral particle gradients: 10 µm – m
CONCLUSIONS

- control of the reactive species delivery
- understanding of the process chain leading to the therapeutical effects
- optimisation of the applications (dose?)