Low-temperature atmospheric-pressure plasmas as a source of reactive oxygen and nitrogen species for chronic wound disinfection

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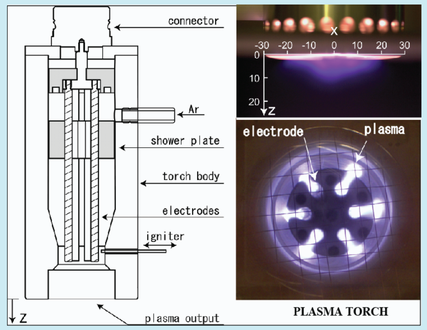
Plasma and Apparatus

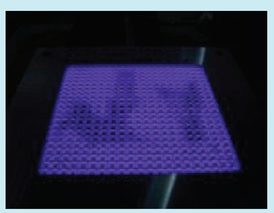
Plasma (*the fourth state of matter*) is an ionized gas that contains ions, electrons, neutral and excited-state particles. Plasma comprises more than 99% of visible universe. Low-temperature plasmas can provide a source of biologically active particles (e.g. reactive species) for biomedical applications. Gases can be ionized using thermal, electrical energy, or energy of photons (UV radiation or intense visible light). To generate low-temperature atmospheric-pressure plasmas we are using energy of electric discharge. Composition and density of reactive species can be controlled by the frequency of applied voltage, pulse operations, by changing the gas environment, gas temperature, or by using a catalyst.

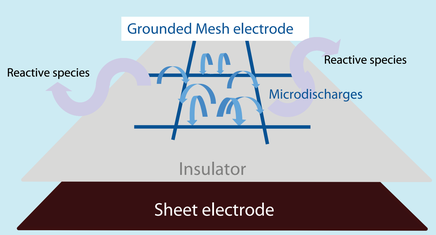
Argon plasma



In this device, an electric discharge is generated in argon atmosphere. Ionized argon interacts with ambient air producing reactive oxygen and nitrogen species. The concentration and composition of reactive species can be regulated by argon flow rate and by supplying a secondary gas to the discharge area. One advantage of using noble gases for producing plasmas is that they are not aggressive toward tissue. Besides, argon is relatively easy to ionize.

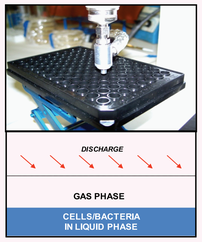
Air plasma





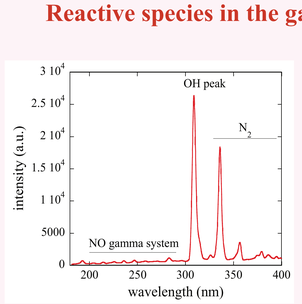
The plasma is generated in air by multiple microdischarges in the strong field produced around the wires. Nonequilibrium chemistry produces reactive oxygen and nitrogen species which rapidly diffuse toward the target. The dosage of reactive species is regulated by voltage and treatment time. The device is designed for disinfecting relatively large surface area.

Experiment (Ar plasma)



Cells and tissue are separated from the plasma generation area by two phases, gas phase and liquid phase (*e.g.* media, PBS, wound fluid). Complex reactions involving reactive oxygen and nitrogen species occur in both phases. There are several sources of reactive species in the liquid phase including diffusion from the gas phase (NO, NO_2, $ONOO^{\cdot}$, $O_2^{\cdot}$, O_3), liquid ionization (*e. g.* H^+, $^{\cdot}OH$, H_2O_2) by the ion bombardment and UV radiation excited by metastable-state molecules and atoms.

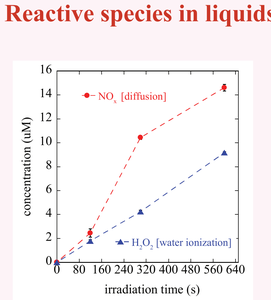
Reactive species in the gas phase



NO: < 1 ppm
NO_2: < 6 ppm
O_3: ~900 ppb

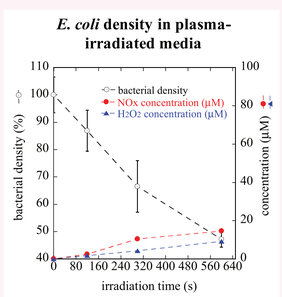
Optical emission spectrum in the UV range produced by argon plasma

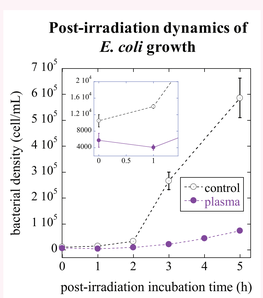
Reactive species in liquids

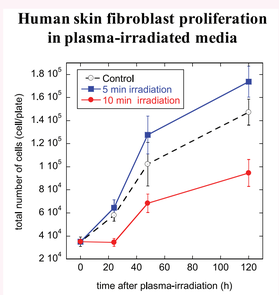


Relative contribution of the diffusion from the gas phase and liquid ionization

Effects of plasma-generated reactive species on bacteria and human cells







Reactive species generated by argon plasma have bactericidal and a long-term (at least five hours) bacteriostatic effects. Plasma irradiation can not only decrease bacterial load in the wound area, but also control bacterial growth between two treatments. Plasma-generated reactive species have dosage-dependent effects on human cells. We observed induction of skin fibroblast proliferation at low (5 min treatment) and inhibition at high (10 min treatment) dose. These doses caused no significant alterations of cell viability and migration.

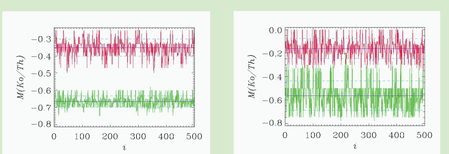
Clinical study

Bacterial density reduction in plasma-treated wounds

Microwave Plasma Steriliser ß – argon plasma device for chronic wound disinfection

Clinical study Phase II. Irradiation with argon plasma has been tested for disinfection of chronic wounds as a treatment supplementary to the standard wound care. Plasma treatment showed to be effective against gram-negative and gram-positive bacteria including the methicillin-resistant strain of *Staphylococcus aureus* (MRSA). Bacterial density was evaluated in 291 samples from plasma-treated and control wound areas from 37 patients. These measurements show 34% ($p < 0.0001$) higher bacterial load reduction in wounds treated with plasma during five minutes per day and 40% in wounds treated with plasma during two minutes per day in comparison with control wounds that received a standard treatment.

5 min treatment 2 min treatment



Bacterial density in plasma-treated vs. control wounds. Bootstrap validation test.

Summary

Results of this study demonstrate that low-temperature atmospheric-pressure plasma is a promising tool for chronic wound disinfection. In addition to bactericidal effect, low doses of reactive species produced by plasma can stimulate cell proliferation and enhance the wound healing process. In future, plasma can be used as a source of reactive species for regulating various pathophysiological processes.