Trends in Biotechnology

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Frugal Biotech Applications of Low-Temperature Plasma

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Gas discharge low-temperature air plasma can be utilized for a variety of applications, including biomedical, at low cost. We term these applications 'frugal plasma' – an example of frugal innovation. We demonstrate how simple, robust, low-cost frugal plasma devices can be used to safely disinfect instruments, surfaces, and water.

Motivation for Frugal Plasma Innovation

Among the fastest growing current applications of low-temperature (cold) plasmas is the field of medicine, including hygiene. Starting from their capability to kill microbes and their applicability to disinfect water, air, medical instruments, and heat-sensitive materials, to the direct applications on teeth, skin, or wounds, or for shrinking tumors as potential cancer therapies, the biomedical applications of cold plasmas established the new domain named 'plasma medicine'. The key question of how to design and run the plasma sources for specific biomedical purposes remains a challenge, because the physical and chemical effects of different plasma sources are not fully understood, and the mechanisms by which plasma affect the biological liquids, cells, and tissues are not well established [1].

The primary focus of this article is to describe a class of simple, inexpensive cold plasma devices operating in air, sometimes using small amounts of water that can be exploited for applications such as biocides, disinfectants, sterilization, and antisepsis, among others. The simplicity and relatively low cost of this class of plasma devices imply that they can be thought of as a kind of 'frugal technology'. We suggest that this novel perspective might open up new applications for atmospheric air plasmas. The idea of making a technology simpler with no 'frills' to render it inexpensive and potentially more robust while still meeting important social and environmental needs has been referred to as 'frugal innovation' or 'frugal engineering' [2,3]. One guiding principle in frugal innovation and engineering is the Indian/Pakistani concept of *Jugaad*, sometimes defined as 'a creative or innovative idea providing a quick, alternative way of solving or fixing a problem' [4]. Of course, any viable and successful technology must meet not only minimal technical specifications but must also satisfy numerous other constraints, including safety, cultural, and local economic issues, etc. Ideally, the technology must also be scalable so that significant numbers of people are served.

Air plasma technology fits naturally into the context of frugal innovation. Frugal plasma sources can be powered by inexpensive, portable solar energy panels (e.g., www.wecaresolar.org). Such installations could charge batteries able to power widely available low-cost small neon-sign transformers as high-voltage power supplies for several hours. Dielectric barrier discharge (DBD), corona discharge, or spark discharge as plasma sources can easily utilize robust but inexpensive ceramics, syringe needles, or automobile spark plugs as electrodes. Using only little electricity, ambient air, and water, the frugal plasma sources can produce ozone for antisepsis or water disinfection, or strongly bactericidal nitrogen oxides to sterilize medical instruments or make antimicrobial plasma-activated water (PAW).

Frugal Plasma Examples

We describe here a few examples of simple, low-cost, and robust plasma sources for biotechnology that utilize electrical discharges in ambient air to create various potentially useful chemical species, such as ozone or nitrogen oxides, sometimes mixed into water, to induce antimicrobial, disinfection, and therapeutic effects.

Ozone Generators for Water Disinfection or Wound Healing

Ozone (O_3) generation by DBD or corona discharge, invented by Siemens in 1857, has been exploited commercially for many years and is now a mature technology used typically in large industrial scales. O_3 dissolved in water is one of the strongest antimicrobial agents known. It has been pointed out that under conditions in which O_3 is formed in air DBD plasmas and dissolved in water, it can (in some cases) be responsible for most of the observed (very rapid) antimicrobial effects in PAW (e.g., [5]). Indeed, there are many small and low-cost ozone generators on the market now (e.g., http://www.ozonesolutions.com).

Recently, Kim and colleagues [6] reported a breakthrough in the design of ozone-creating atmospheric pressure microplasma devices (e.g., http://engineering.illinois.edu/news/article/16847). Some of the ideas regarding frugal plasma could certainly be applied to novel ozone-generating plasma devices based on DBDs or corona discharges in air that can be exploited for water disinfection and medical instrument sterilization by ozonated water.

In the context of wound healing, recent reports show that various plasma devices are capable of promoting wound healing [7]. The mechanisms responsible for these effects are not yet fully understood, but they probably include the effects of reactive oxygen species, including ozone.

Portable Plasma Sources for Surface Disinfection and Wound Healing

In air plasmas, it is known that low gas temperatures ($<$350 K) and low power deposition, as well as relatively dry air, favor the formation of O_3 over nitrogen oxides and nitric acid. When depositing more power in ambient air, nitrogen oxides and nitric acid formation will compete with O_3 and the plasma electrons dissociate ambient water molecules to produce highly reactive OH (hydroxyl) radicals that consequently recombine to make hydrogen peroxide (H_2O_2) [8]. At still higher powers, nitric oxide and its related species (e.g., nitrite, NO_2^-) are created in significant quantities in air plasmas and these compounds have been shown to be effective, for example, in killing fungi growing under toenails [9] and treating infected wounds [10].

We present an example of a frugal portable air plasma ‘corona pen’ that employs a neon-sign transformer with a rectifier and voltage multiplier to power a DC-positive streamer or negative corona discharge on a needle electrode in ambient air [11] that generates O_3, NO_x, and OH radicals at very low power (<1 W). The streamer corona, especially in combination with water, introduced, for example, by electrospray, can be strongly antibacterial for disinfection of surfaces and even inactivation of antibiotic-resistant biofilms [11]. Figure 1 shows a photograph of a positive streamer corona discharge on this portable air plasma pen and examples of a direct treatment of bacteria on agar plates by this plasma source. Such sources have potential for medical instrument disinfection or direct *in vivo* application for wound healing or dentistry. An example of a portable air plasma device that could serve in this way is the battery-powered, handheld ‘plasma flashlight’ [12]. Devices such as this could be manufactured for less than \$100 with power dissipation typically less than several watts. Although yet to be demonstrated in clinical practice, it seems likely that they would be useful in both point-of-care applications and centralized facilities.

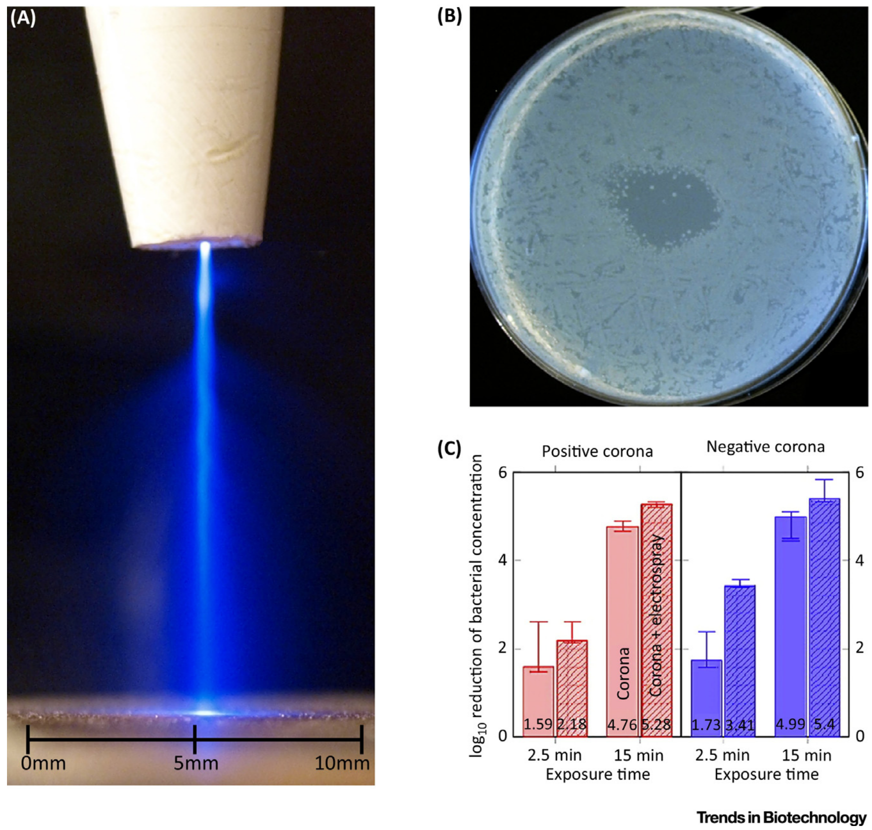

Figure 1. Portable ‘Corona Pen’ and Its Antibacterial Effects. (A and B) Photographs of the positive air streamer corona discharge generated by the portable air plasma pen and its antibacterial efficacy expressed as decontaminated area of *Escherichia coli* grown directly on agar plates. (C) Logarithmic reduction of bacterial concentration in *E. coli* biofilm grown on glass by positive and negative corona discharges, without and with the effect of water electrospray on the sample. Reproduced, with permission, from [11].

Antimicrobial NO_x Generation for Medical Instrument Sterilization

As noted earlier, if the power dissipation in air plasmas is further increased, the O_3 production will be completely suppressed and the NO_x production will dominate. It is possible to create powerful antimicrobial concentrations of NO and especially NO_2 in such plasmas [13,14].

One example of this approach is the ‘NOxBox’, which uses a hybrid glow-spark air discharge using a regular automobile spark plug, with relatively small amounts of electrical power from inexpensive power supplies, to produce antimicrobial NO_x gases (Figure 2). This frugal air plasma source dissipates approximately 30 W in a 1-L confined volume to generate 2000–3000 ppm NO_x in 5 min, with the NO_x generation rate of over 10^{16} NO_x molecules/J. Approximately 80–90% of the NO_x is in the form of NO_2, which is the dominant antimicrobial component. The strong antimicrobial action of the NO_x mixture after several minutes of plasma operation was demonstrated by measuring rates of *Escherichia coli* disinfection on metal surfaces and in water exposed to the plasma volume [14]. Scale-up to fill a 5–10-L container with useful concentrations of NO_x that might be used, for example, for medical instrument sterilization, is relatively straightforward.

Concluding Remarks on Frugal Plasma Sources

There are many other potential applications for the kind of frugal plasma devices presented here. One example is PAW prepared by water interaction with air plasma. PAW contains large concentrations of reactive oxygen and nitrogen species (RONS), such as H_2O_2, nitrates and

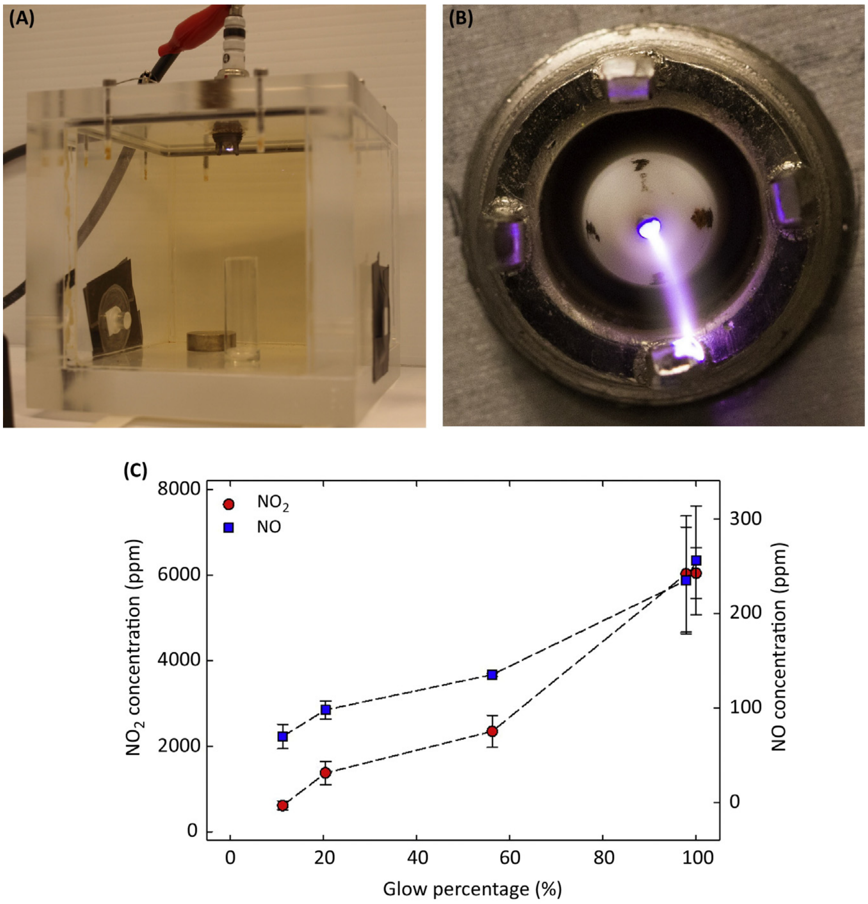

Figure 2. 'NOxBox' for Medical Instrument Sterilization by Antimicrobial NO_x. (A and B) Photographs of the *NOxBox* with the detail of the hybrid glow-spark air discharge on a spark plug. (C) Temporal evolution of *Escherichia coli* log reductions on stainless steel surfaces and in aqueous saline solution in the *NOxBox* powered by the quasi-sinusoidal AC waveform from neon-sign transformer. Reproduced, with permission, from [14].

nitrites, and peroxynitrites. Some of these compounds have been used for food preservation for many centuries. Many RONS have potent antibacterial activity against a range of pathogens. In particular, NO and NO_2^- (nitrite) have various physiological functions in organisms and have been extensively explored for various therapeutic applications [15].

One of the key advantages of air plasma-based systems to generate these species is that one needs only electricity and ambient air (and water in some cases). Many situations can be envisioned in which supplies are difficult or impossible to obtain, including natural disasters and various other serious social disturbances such as armed conflicts. A portable frugal plasma system could be lifesaving in this context, assuming that small amounts of electrical power are available, such as those provided by solar charging of batteries or other sources.

It must be acknowledged that safety issues are important when using plasma devices that create large concentrations of antimicrobial, but also potentially toxic, compounds. In the case of antimicrobial O_3 or NO_2 gases, for example, chronic exposures can lead to various adverse health effects. Care must therefore be taken to minimize the risks for toxicity in whatever application is chosen for frugal plasma devices.

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