International Journal of Antimicrobial Agents 43 (2014) 508–517

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Review

Atmospheric pressure plasmas: Infection control and bacterial responses

Anne Mai-Prochnow[a,*], Anthony B. Murphy[a], Keith M. McLean[b], Michael G. Kong[c], Kostya (Ken) Ostrikov[a]

[a] CSIRO Materials Science and Engineering, P.O. Box 218, Lindfield, NSW 2070, Australia
[b] CSIRO Materials Science and Engineering, Bayview Avenue, Clayton, VIC 3168, Australia
[c] Frank Reidy Research Center for Bioelectrics, Old Dominion University, Suite 422, 4211 Monarch Way, Norfolk, VA 23529, USA

ARTICLE INFO

Article history:
Received 11 December 2013
Accepted 27 January 2014

Keywords:
Atmospheric pressure plasma
Bacterial resistance
Biofilms

ABSTRACT

Cold atmospheric pressure plasma (APP) is a recent, cutting-edge antimicrobial treatment. It has the potential to be used as an alternative to traditional treatments such as antibiotics and as a promoter of wound healing, making it a promising tool in a range of biomedical applications with particular importance for combating infections. A number of studies show very promising results for APP-mediated killing of bacteria, including removal of biofilms of pathogenic bacteria such as *Pseudomonas aeruginosa.* However, the mode of action of APP and the resulting bacterial response are not fully understood. Use of a variety of different plasma-generating devices, different types of plasma gases and different treatment modes makes it challenging to show reproducibility and transferability of results. This review considers some important studies in which APP was used as an antibacterial agent, and specifically those that elucidate its mode of action, with the aim of identifying common bacterial responses to APP exposure. The review has a particular emphasis on mechanisms of interactions of bacterial biofilms with APP.

1. Introduction

Several infectious diseases, including lower respiratory infections, diarrhoeal diseases and HIV/AIDS, are consistently among the major causes of death in the world according to the World Health Organization (WHO) (http://www.who.int/mediacentre/factsheets/fs310/en/index.html). Whilst there are a range of antimicrobial measures available, combating infections is still challenging because of ever-increasing microbial resistance. Bacterial resistance to antibiotics, in particular in hospital settings, results in loss of human life and increased costs. Whilst antibiotic resistance in bacteria occurs naturally to some extent [1,2], inappropriate use and overuse of these drugs has increased this threatening process at an alarming rate [3]. To reduce antimicrobial resistance and to succeed in combating infection it is necessary both to optimise current antibiotic use and to search for better antimicrobial measures that are not subject to evolving resistance.

The techniques developed to eradicate unwanted bacteria include the use of antibiotics, heat, pressure, ultraviolet (UV) and, more recently, antimicrobial nanoparticles, nanostructured surfaces, antibacterial peptides and cold atmospheric pressure plasma (APP) (Table 1) [4–8].

Plasma, the fourth state of matter, is ionised gas and can be generated using a range of gases or gas mixtures, typically argon, helium, nitrogen, air or oxygen. Plasma generated in air consists of a reactive mix of atoms, excited molecules, charged particles, reactive oxygen species (ROS), reactive nitrogen species (RNS) and UV photons, all of which may contribute to its antibacterial properties (Fig. 1).

APP can be generated by a range of devices and has been shown to inactivate bacteria [11], cancer cells [12,13], fungi [14,15], spores [16], parasites [17], phages and viruses [18,19] (Fig. 2). It is being used in applications such as surface sterilisation [15,22], food decontamination [23], dermatology [24] and dentistry [25,26].

APPs have come into the spotlight as an effective alternative to traditional antibiotics for non-systemic infections, as plasma treatment shows remarkable effectiveness against a range of micro-organisms, including antibiotic-resistant biofilm-forming strains and spores (Table 2). However, recent studies suggest that

* Corresponding author. Tel.: +61 2 9490 8451.
E-mail addresses: anne@amp-bio.com, anne.mai-prochnow@csiro.au (A. Mai-Prochnow).

http://dx.doi.org/10.1016/j.ijantimicag.2014.01.025
0924-8579/

Table 1
Comparison of atmospheric pressure plasma (APP) features with other infection control measures.

Method	Advantages	Disadvantages
APP	Short treatment time Highly effective Very limited side effects Applied locally	Unspecific (would kill local normal flora) Only limited internal use
Antibiotics	Established dosage available Different antibiotics for different bacteria	Many possible side effects Resistance of bacteria Long treatment time
Ultraviolet	Simultaneous treatment for large surface areas	Damage to human cells
Heat	Low cost	Not suitable for direct medical applications
Nanoparticles	Highly localised treatment Target-specific Delivery to specified areas	Difficult to manufacture Nanosafety (toxicity) Internalisation on cells
Surface chemicals (e.g. H_2O_2, ethanol)	Low cost	Only short-term effect

the bacterial response to APP treatment varies depending on a range of factors, including bacterial species, growth phase and mode of growth as well as the plasma characteristics.

Here we review recent advances in the use of APP to eradicate bacteria. The mode of action of APP is discussed and possible bacterial response mechanisms to APP exposure are considered.

2. Atmospheric pressure plasma use in infection control

2.1. Atmospheric pressure plasma-generating devices

Plasmas suitable for use in infection control are cold (close to room temperature) and generated at atmospheric pressure. Plasma-generating devices used for sterilisation and disinfection include remote plasma jets or plumes and direct plasma sources as well as hybrid devices (Fig. 1). Remote plasmas are characterised by closed-circuit loops and a self-contained electrode system, with plasma generated in a defined cavity and then transferred into an ambient environment by a gas flow. When a plasma jet is used, the treated surface (e.g. infected skin or a medical device) is not in direct contact with the plasma but instead with the plasma effluent (Fig. 1A). In contrast, in a direct plasma source, high-voltage cold plasma is generated between a dielectric surface covering the electrode and the biological sample that serves as a second electrode, giving it direct contact with the plasma (Fig. 1B) [36]. Direct treatment brings charged energetic particles in contact with the treated sample/bacteria and a faster killing of bacteria is observed [37].

APPs can be generated by applying a wide range of frequencies to the gas mixture, from direct current to radiofrequency, and GHz range microwave sources. When designed and controlled appropriately, APP is safe to touch and can be used on sensitive materials (e.g. living tissues) without causing heating, electric shock or pain.

In addition to devices suitable for skin and surface use, a 'plasma gun' has been developed in which plasma is generated inside narrow, flexible, dielectric capillaries of several tens of centimetres in length [38]. This design has obvious potential for delivering plasma endoscopically and opens a range of possibilities for use during surgery.

Fig. 1 shows two plasma-generating devices commonly used in biomedical applications. The main differences between plasma sources are the power supply, electrode design and choice of gas. It has been shown that operation of the same plasma source with two different power supplies generating voltage pulses of different duration affected the bacterial killing efficiency [39]. In these experiments, plasma generated with a short pulse (first half-cycle length 30 ns) and high peak voltage (14.1 kV) was more efficient in killing *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Microsporum canis* grown on agar plates compared with when the plasma was generated with a long pulse (first half-cycle length 2 μs) and lower peak voltage (10.3 kV). This may be because the electrons produced are accelerated by a larger electric field in high peak voltage and are therefore more energetic. These energetic electrons can trigger chemical reactions with higher energy threshold and as such enable additional and often

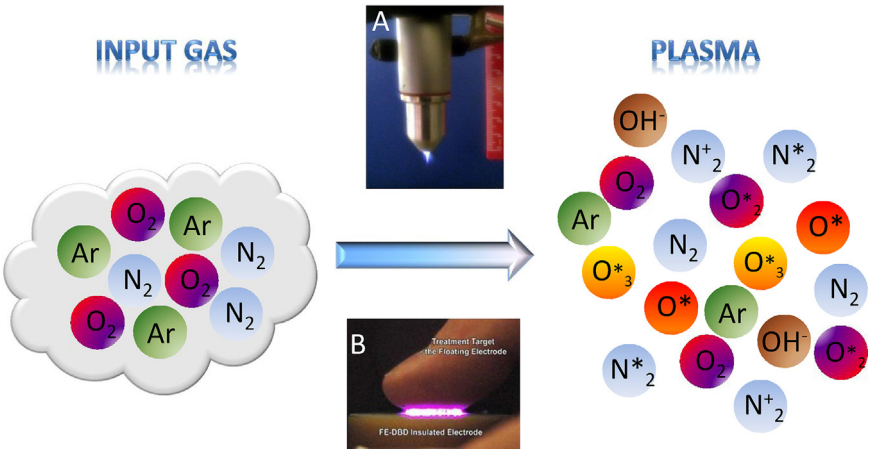

Fig. 1. Atmospheric pressure plasma devices and their output. A gas is fed through an electric current and plasma is produced with a mix of reactive species originating from the input gas as well as the ambient air. Plasma is used for treatment either (A) as an indirect effluent such as the atmospheric pressure plasma jet (kINPen 09; INP Greifswald, Germany) [9] or (B) as a direct floating-electrode dielectric-barrier discharge (FE-DBD) plasma [10].