Current Applied Physics 14 (2014) S142–S148

Contents lists available at ScienceDirect

Current Applied Physics

journal homepage: www.elsevier.com/locate/cap

Inactivation of *Propionibacterium acnes* and its biofilm by non-thermal plasma

Anser Ali [a,b], Yong Hee Kim [b,c], Jin Young Lee [b,c], SeungHyun Lee [a,b], Han Sup Uhm [b,c], Guangsup Cho [c], Bong Joo Park [b,c,*], Eun Ha Choi [a,b,c,*]

[a] Department of Plasma Bioscience and Display, Kwangwoon University, 20 Kwangwoon-gil, Nowon-gu, Seoul 139-701, Republic of Korea
[b] Plasma Bioscience Research Center, Kwangwoon University, 20 Kwangwoon-gil, Nowon-gu, Seoul 139-701, Republic of Korea
[c] Department of Electrical and Biological Physics, Kwangwoon University, 20 Kwangwoon-gil, Nowon-gu, Seoul 139-701, Republic of Korea

ARTICLE INFO

Article history:
Received 5 August 2013
Received in revised form
20 November 2013
Accepted 2 December 2013
Available online 23 January 2014

Keywords:
Propionibacterium acnes
Biofilm
Inactivation
Non-thermal plasma
Reactive species

ABSTRACT

Propionibacterium acnes (P. acnes) is an opportunistic gram positive pathogen which has become an important source of various surgical implant associated infections including artificial joints, shunts, heart valves and catheter's infections. In addition, *P. acnes* can form biofilm which may enhance the complications even more. Even though it is susceptible to most of the antibiotics but still hard to remove and in severe cases removal of device is suggested. This makes the failure of implants. These problems prompted us to find more efficient method to sterilize these contaminations. Non-thermal plasmas primarily generate reactive species and recently have emerged as an efficient tool for medical applications including sterilization. Therefore, in this study we evaluated the inactivation ability of two different plasma jets, non-thermal annular plasma jet (NAPJ) and non-thermal soft plasma jet (NSPJ) for *P. acnes* in planktonic state and biofilm state. And, we found that both plasma devices showed considerable inactivation potential in planktonic *P. acnes* and *P. acnes* biofilms. Especially, NSPJ showed better inhibitory effect in shorter exposer time than NAPJ which might be because of close exposure to plasma generated reactive species. Moreover, we found that intracellular and extracellular reactive species concentrations are correlated with plasma treatment time, which suggest their critical role in microbial inhibition.

In conclusion, our study suggests that plasma technology may also be used to overcome the biofilm contamination problems associated with biomaterials including surgical devices.

1. Introduction

Biofilm is a community of bacteria attached to an inert or living surface, enclosed in self-made matrix usually consisting of polysaccharide and having the ability to secrete various enzymes and virulence factors [1,2]. People may be exposed to bacteria by medical devices during implantation and they suffer by infections mainly because of bacterial attachment and aggregation on medical devices, release of their end-toxins, their tolerance to antibiotics and compromised host body defense system [3,4].

The reduced antimicrobial susceptibility is another dilemma in the way of sterilization and is also linked with the ability of bacteria to develop biofilm. It is proved that biofilm is a natural survival strategy of bacteria and serve as reservoir of infection for host. According to national institute of health recent public announcement, over 60% of all bacterial human infections are caused by biofilms which might be the result of their inherent tolerance to antibiotics and host body defense system [3,4].

Propionibacterium acnes (*P. acnes*), mainly skin inhabitant gram positive bacteria, can be found in other body parts such as intestine, oral cavity, external ear canal and conjunctiva of the eye [5]. This non-spore forming, rod shaped opportunistic pathogen is well known for skin acne but can also participate in other disorders such as prostate inflammations, leading to wide range of complications for example, cancer, hyperostosis, synovitis, osteitis, pustulosis, sciatica and sarcoidosis. Moreover, it is frequently diagnosed in postoperative infections of mouth, brain, eye, bones as well as infections associated with implants [5] such as prosthetic joints especially shoulder implants [6–8], breast device related [9,10] and electrophysiological cardiac devices [11] including ventriculoperitoneal shunts and heart valves [5,12].

* Corresponding authors. Department of Plasma Bioscience and Display, Kwangwoon University, 20 Kwangwoon-gil, Nowon-gu, Seoul 139-701, Republic of Korea.
E-mail addresses: choipdp@gmail.com, parkbj@kw.ac.kr (B.J. Park), choipdp@gmail.com, ehchoi@kw.ac.kr (E.H. Choi).

1567-1739/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.cap.2013.12.034

In anaerobic conditions, *P. acnes* can survive as long as 8 months *in vitro* without sub-culture [13], which represents their long time persistence ability even in low oxidation state. In addition they can live among macrophages [14] and can grow well in plasma deficient environment inside the human body such as implants. These advantages help them in long time subsistence and make them a powerful threat for foreign biomaterial contamination. Particularly, the development of bacterial biofilm is one of the leading sources of biomaterial contamination and implant associated infections [15]. It has been proved that *P. acnes* retain the ability to form biofilm *in vitro* as well as *in vivo* [16,15]. *P. acnes* is as frequent source of implant biomaterial contamination as *Staphylococcus aureus* however, dramatically under estimated previously. *P. acnes* showed more antibiotic resistance particularly in biofilm than in planktonic phase [17]. This fact has been verified, as Tunney et al. [18]; demonstrated that *P. acnes* can form biofilm on gentamicin-loaded bone cement even when supplemented with antibiotics in medium. The significantly reduced antimicrobial susceptibility of biofilms, release of end-toxins, tolerance to host immune system result in chronic and persistent severe contaminations that are difficult to remove without surgical elimination of implants.

Non-thermal plasma has been considered as an effective method of sterilization because of its ability to produce, radicals, ions and reactive species important for sterilization and, for various other reasons such as low cost, easy handling, and most importantly not leaving toxic effects after treatment. Therefore, we accessed the efficacy of our self-designed two non-thermal plasma's to remove and sterilize the *P. acnes* and its biofilm.

2. Materials and method

2.1. Microorganism

Propionibacterium acnes KCTC 3314 was obtained from Korean Collection for Type Culture (KCTC). Prior to experiment, the bacterial strain was stored at −70 °C in deep freezer. For experiment, the bacterial strain was grown on the reinforced culture media (RCM; Becton, Dickinson and Company, Sparks, MD, USA) at 37 °C (5% CO_2 and anaerobic conditions) for 3–5 days.

2.2. Plasma devices

Two kinds of plasma devices were used which are named as non-thermal annular plasma jet (NAPJ) and non-thermal soft plasma jet (NSPJ). Both were designed by our own laboratory. The sample temperature after maximum plasma treatment used for each plasma device was observed bellow 40 °C.

NAPJ used in this study is shown in Fig. 1(a). It mainly consists of power supply, gas regulator and tube shaped body (124 mm in length). This body mainly comprises of two copper electrodes (inner and outer). Inner electrode is 10.2 mm in diameter, 21.4 mm in length and it is located at distal end of inner quartz tube of 11.2 mm diameter. Outer electrode lies on outer quartz tube (19.2 mm in outer diameter) and form a 1.2 mm thin ring of 4.5 mm broad copper band above the inner electrode. Argon gas is injected from top through a small gas inlet and it flows at the rate of 1 L per minute (lpm) between inner and outer quartz tubes and approaches down to the electrodes level. When electrodes are connected to 10 mA current and 3 kV voltages, keeping 25 kHz frequency, then plasma is generated around inner electrode. Generated plasma cannot come in direct contact with sample because of outer quartz tube that was extended 42.2 mm in front as shown in Fig. 1(a). The distance between sample and device was fixed as 3–4 mm.

The other device used in this study is NSPJ and previously explained by our research member Kaushik et al. [19]; in detail. Briefly, it consists of three major components called high power voltage supply, electrodes and dielectrics as shown in Fig. 1(b). To supply high voltage power, commercial transformer was used and primary voltage was controlled by voltage controller. The plasma

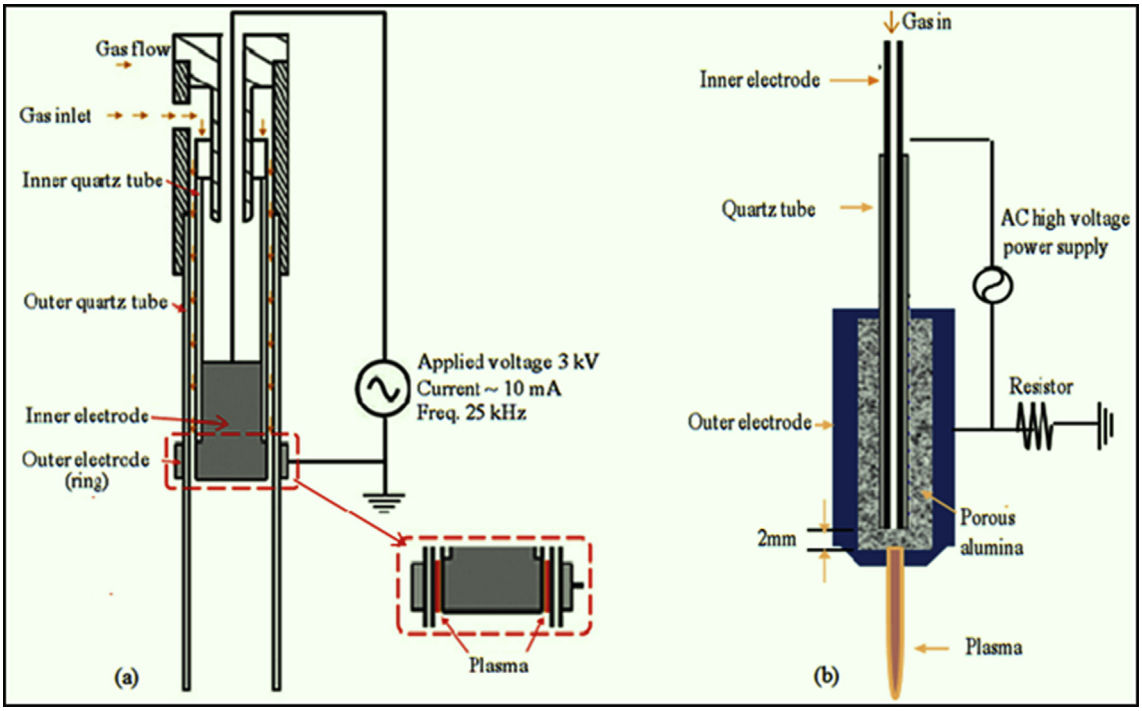

Fig. 1. Schematic diagram of both non-thermal plasma devices. (a) Annular plasma jet (NAPJ) and, (b) non-thermal soft plasma jet (NSPJ). Argon and air was used as working gas in NAPJ and NSPJ, respectively at the rate of 1 lpm throughout the experiment.