September 8, 2018

Pipath Poramapijitwat  
Nanoscience and Nanotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand

Dear Mr. Pipath Poramapijitwat,

On behalf of the organizing committee, I would like to inform you that your abstract “EFFECT OF DIELECTRIC BARRIER DISCHARGE PLASMA JET (DBDJ) ON HUMAN DERMAL FIBROBLAST ADULT (HDFA) CELLS AS IN VITRO CONTAMINATED WOUND HEALING MODEL” has been accepted as an oral presentation at the International Conference on Radiation and Emission in Materials (ICREM-2018), which will be held in Chiang Mai, Thailand, during November 20-24, 2018.

The ICREM-2018 is foreseen to cover the radiation and emission phenomena in its natural combination in the range from accelerated ionizing particles to THz electromagnetic radiation, building on the similarity of the basic principles and benefitting from the variety of applications. More information can be found via http://www.science.cmu.ac.th/ICREM-2018/index.php.

This letter serves as an official invitation from the organizing committee to have you present your research at the conference. We also hope this letter may serve as an official conference document when you apply for your travel approval by your institution as well as the required visa document to enter Thailand. We look forward to your participation and presentation.

Please note that your abstract might be modified to fit to the conference format and the presentation session might be assigned to be in a different topic from the submitting.

The conference registration is open. Please note the early registration deadline is September 30. Chiang Mai is a busy tourist destination, so you should consider to register as soon as possible. Late or on-site registration will be possible at a rate of USD500 and USD250 for conference attendees and accompany persons, respectively. These fees will cover all events of the conference program but do not guarantee the accommodation and exclude its cost.

We look forward to welcoming you to this very beautiful tourist city in November. Please do not hesitate to contact us if you have any questions.

Sincerely,

Dheerawan Boonyawan, PhD  
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Plasma and Beam Physics Research Facility  
Department of Physics and Materials Science  
Chiang Mai University  
Chiang Mai 50200
International Conference on Radiation and Emission in Materials

20-23 November 2018
Holiday Inn Hotel, Chiang Mai, Thailand

InnoPlasCM
Radiation and Emission in Materials

E-proceedings of International Conference on Radiation and Emission in Materials (ICREM 2018)

Holiday Inn, Chiang Mai, Thailand
November 20-23, 2018
Preface

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Editorial

PROCEEDINGS OF THE INTERNATIONAL CONFERENCE ON RADIATION AND EMISSION IN MATERIALS (ICREM 2018)

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It is our pleasure to publish the e-proceedings of the International Conference on Radiation and Emission in Materials (ICREM), which took place between 21 – 23 November 2018.

The conference covers the radiation and emission phenomena in its natural combination in the range from accelerated ionizing particles to THz electromagnetic radiation, building on the similarity of the basic principles and benefitting from the variety of applications. The mission of the ICREM is to bring new perspectives to the field of radiation phenomena in advanced materials by providing a forum for researchers and industrialists for exchanging data and ideas including i) Radiation phenomena with ion beams, ii) Electrons and gamma-rays in science and technology, iii) Light-matter interactions in fundamentals and applications, iv) Radiation and emission at IR and THz range, v) Advanced emitting devices, vi) Nuclear radiation for smart materials, vii) Plasma technology and viii) Solar cells.

The focus is on advances in fundamental understanding as well as its exploitation in modern instrumentations (scientific, technological, medical, etc.) and technologies/devices (nuclear radiation, plasma technology, radiation detectors, photovoltaics, light emitting diodes, lasers, single photon emitters, etc.)

The first conference of ICREM held in 2018 is a limited-scale event. About 100 abstracts have been submitted to be presented in the conference. Since a variety of radiation effects have been proposed to the conference, they are plenty of topics for the valuable discussion. The conference objective was achieved by contributions of our participants as well as the reviewers.

The conference was held in a single conference room without parallel sessions, contained 10 sessions with 18 invited talks. Furthermore, 44 abstracts were presented in the conference as 19 contributed talks as well as 25 poster presentations. The presentation during the poster session came along with welcome party that makes the discussion becomes more relaxed after a long day.

During the conference one poster prize for student who had exceptional poster-presentation was awarded to S. Pakluea (Chiang Mai University) for the presentation entitled “THz Transition Radiation from Short Electron Bunches; Polarization Measurement and Imaging Experiment”.

The cooperation of local staffs, local committees and international committees is a main organization for accomplishing the conference. The presentation program was arranged with an assist of international committee as well as local staffs. For the communication between participants and organizers, we would like to thank the IT service staffs at Faculty of Science, Chiang Mai University for a friendly-use webpage as well as the announcement of any information through the conference.

We anticipate launching the bigger-scale regular ICREM series from Fall 2019, including Industrial Forum, Intensive Course for Young Researchers, Carrier Center, etc. Thailand, by its own, is one of the most frequent/attractive destinations worldwide and as such probably does not require additional motivation for its attractiveness. Importantly, Chiang Mai - as a regular ICREM location - exhibits rather unique and in the first glance ‘excluding’ combinations of

- ultimate remoteness but relatively easy accessibility for overseas travelers,
- being a cross-civilization point for centuries in regional history but remaining a small megapolis-free problem town,
- being in proximity of a hot climate zone but typically exhibiting warm comfortable weather in mid-November,
- being surrounded by wats and rural villages but hosting one of the best Thailand universities – Chiang Mai University (CMU) – and in particular the ion beam and plasma physics center of excellence having a wide international collaboration network.
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PLASMA EFFECT OF DIELECTRIC BARRIER DISCHARGE PLASMA JET (DBDJ) ON HUMAN DERMAL FIBROBLASTS ADULT (HDFa) CELLS: IN VITRO CONTAMINATED WOUND HEALING MODEL

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Abstract

The dielectric barrier discharge plasma jet (DBDJ) was used to study the effect of plasma radicals on S. aureus, P. aeruginosa, biofilm and Primary Human Dermal Fibroblasts adult (HDFa) cells. The He plasma was generated by high-voltage DC pulse. Plasma radical species were observed. The N2, NO, He and OH radical groups were found. The intensity of radical in plasma depends on the repetition rate applied by the plasma system. After treatment with plasma at 0.50 watt and exposure time at 60 s, the result showed that it was the best condition for killing bacteria up to 100%. Therefore, this condition was chosen to study the effect of plasma on biofilm and HDFa cells. The result showed that DBDJ had high efficiency to removed biofilm. After plasma treatment the shape of HDFa cells was unchanged. The results of Mune Count & Viability Assay Kit and Muse Annexin V & Dead Cell Assay Kit showed plasma had slightly effect on HDFa cell viability. Therefore, the DBDJ has high efficiency to kill bacteria, destroy biofilm and assist in contaminated wound healing without damage to skin cells.

Keywords: Dielectric Barrier Discharge Plasma Jet; Human Dermal Fibroblasts adult cells; Bactericidal; Wound Healing; Bacterial Biofilms

2. Experimental Procedure

The DBDJ plasma device operated in a pure He mode, described elsewhere [3]. The emission spectrum of this DBDJ was obtained by broadband CCD spectrometer (Fiber Optic Spectrometer: AvaSpec-2048). The high voltage waveform was determined using a high-voltage probe (Tektronix, P6015A). The plasma dissipated power was estimated by Lissajous figure [4], where the discharge charge was estimated from the voltage across the 1 nF capacitor measured by a HV probe (Hantek, T3100). The NO and O2 concentration were measured using the two gas detectors (Shenzhen YuanFe Technology): model SKY2000-NO and SKY2000-O2.

Preparation of S. aureus and P. aeruginosa

Bactericidal efficiency was observed by Colony Forming unit (CFU) method. Samples of TISTR 2329 S. aureus and TISTR 2370 P. aeruginosa were obtained from Thailand Institute of Scientific and Technological Research (TISTR). The S. aureus and P. aeruginosa were grown in 5 ml nutrient broth (NB). The grown bacteria was spread onto nutrient agar plates (NA). The DBDJ plasma operated at plasma dissipated 0.27 to 0.50 watt and exposure time 15 to 60 s.

Preparation of Biofilms

The bacterial biofilms both of S. aureus and P. aeruginosa were prepared in NB and then moved to 12-well plate at 1 mL/well on the coverslip glass. The bacteria samples were incubated for 48 hr. After that, plasma exposure on bacteria samples was carried out at plasma dissipated 0.50 watt and exposure time 60 s. Live/Dead assay method with double stain Hoechst 33342 and Propidium iodide (PI) was used. Bactericidal performance of the studied plasma was observed under fluorescence microscope.
Preparation of HDFa
Human Dermal Fibroblasts, adult (HDFa) Cat. no. C-0135C was purchased from Cascade Biologies™ invitrogen cell culture (GIBCO invitrogen cell culture). Effect of DBDJ on HDFa cells was also studied using Live/Dead assay method. In addition, Muse Cell Analyzer with the Muse Count & Viability Assay Kit and Muse Annexin V & Dead Cell Assay Kit was utilized to find survivalability. One-way ANOVA in R-program x64 ver.3.5.1 with R-Studio ver.1.1.456 was used for data interpretation. Post hoc multiple comparison test (Tukey method) was applied at 99% confidence level (α=0.01) [5].

3. Results and Discussion

Plasma properties
The reactive oxygen and nitrogen species (RONS) played an important role in bactericidal [10]. These radicals and electrostatic force of ions break down bacteria cell, DNA damage and charge accumulated leading to cell lysis. The main bactericidal was RONS that could kill bacteria [11, 12].

![Figure 1](image1.png)
Figure 1 The emission spectra of the plasma dissipated power at 0.50 watt.

![Figure 2](image2.png)
Figure 2 The relative of the RONS intensity and the plasma dissipated power.

Figure 3 The relative between NO and O₂ concentration and the plasma dissipated power.

The OES spectrum of DBDJ is presented in Fig. 1. It is found the strongest peak of NO at 297.61 nm, OH at 308.99 nm, N₂ at 337.54 nm and He at 706.54 nm. Fig. 2, intensity of N₂ shows a steady increase with intensity with the plasma dissipated power, while intensity of OH and NO presents a slight increase when the plasma dissipated power increase.

Regarding NO, as seen in Fig. 3, NO concentration exhibits a remarkable increase with the plasma power, which is increased from 1 ppm at 0.25 watt to 5 ppm at 0.5 watt. Whereas, O₂ concentration shows an infinitesimal value less than 0.5 ppm. In biomedical applications, concentration of O₂ and NO should not be higher than 8 hr TWA 0.1 ppm [6] and TWA 25 ppm [7], respectively, otherwise it could adversely affect the respiratory system.

Bactericidal efficiency
Evaluation of antibacterial activity of DBDJ to S. aureus is shown in Fig. 4. It is observed that there is no bacteria inhibition zone in Fig. 4a and 4b, indicating that DBDJ with plasma dissipated power at 0.27 watt and time of exposure of 15–30 s does not exhibit bactericidal manner. However, as seen in Fig. 4c-4h, the bacteria inhibition zone becomes visible and increases with plasma dissipated power and time of exposure. This reveals that DBDJ were potentially effective in suppressing microbial growth when plasma dissipated power and time of exposure was increased. This finding is consistent with bactericidal efficiency which was evaluated by CFU method (Fig. 5). It is seen that bactericidal efficiency increases with both plasma dissipated power and time of exposure.

![Figure 4](image3.png)
Figure 4 The S. aureus bactericidal efficiency of DBDJ, the plasma dissipated power at 0.27 watt and exposure time 15 to 60 s (a, b, c and d), the plasma dissipated power at 0.50 watt and exposure time 15 to 60 s (e, f, g and h).
Figure 5 The percentage of _S. aureus_ bactericidal efficiency by DBDJ.

Bactericidal effect of DBDJ on _P. aeruginosa_ is presented in Fig. 6 and 7. Similar to _S. aureus_ results, plasma from DBDJ can cause apoptosis of _P. aeruginosa_ and bactericidal performance increases with plasma dissipated power and time of exposure. It is found that bactericidal effect of DBDJ on _P. aeruginosa_ shows more effective than that on _S. aureus_. The difference in antibacterial activity of DBDJ on both bacteria could be caused by the difference in the cell wall structure. The outer structure of _S. aureus_ cell wall is multi layers of peptidoglycan which has stronger bonding than _P. aeruginosa_ outermost cell wall of which consists of phospholipids and lipopolysaccharides. Destruction of peptidoglycan through external stresses will lead to cell lysis [8, 9].

![Figure 6](image)

Figure 6 The _P. aeruginosa_ bactericidal efficiency of DBDJ, the plasma dissipated power at 0.27 watt and exposure time 15 to 60 s (a, b, c and d), the plasma dissipated power at 0.50 watt and exposure time 15 to 60 s (e, f, g and h).

![Figure 7](image)

Figure 7 The percentage of _P. aeruginosa_ bactericidal efficiency by DBDJ.

Effect of DBDJ on Bacterial Biofilms

DBDJ plasma operated at 0.5 watt and exposure time 1 min have high ability to eradicate bacteria as show in Fig. 4-7, the Fig. 8 show the fluorescent image. The result has shown this condition have high enough to eradicate bacterial and destroy bacterial biofilm [10] both of _S. aureus_ and _P. aeruginosa_.

![Figure 8](image)

Figure 8 Bacterial biofilms of _S. aureus_ and _P. aeruginosa_ by Live/Dead assay with double stain Hoechst 33342 and Propidium iodide (PI). Control sample _S. aureus_ (a, b and c) and _P. aeruginosa_ (g, h and i). Plasma power at 0.5 watt and exposure time 1 min for removed bacterial biofilm _S. aureus_ (d, e and f) and _P. aeruginosa_ (j, k and l).

Furthermore, the charged particles in plasma played an important role for bactericidal with the rupture of bacteria cells wall [11, 12]. When the charge particles accumulated on outer surface of the membrane being more than the tensile strength of the membrane lead to cell rupture by electrostatic force [13]. CAPPs has the ability to destroy the proteins and enzymes activity into the cell. Most likely the cytoplasm will leaks out the cell through these holes, which was the reason leading the cell death why the cell dies [12, 14].

Cytotoxicity of HDFa Cells

Fig. 9 show the fluorescent image of HDFa cells. After treated HDFa cell with DBDJ plasma dissipated power at 0.5 watt and exposure time 1 min, the Fig. 9j to 9l show the plasma was not damage to the cells.

![Figure 9](image)

Figure 9 HDFa cells by Live/Dead assay with Hoechst 33342 and Propidium iodide (PI). Negative control as 10% FBS+DMEM (a, b and c), positive control as 30% DMSO +DMEM (d, e and f), treatment control as He gas blow (g, h and k) and plasma treated at 0.5 watt in 1 min (j, k and l).
The viability percentage of HDFa cells: negative control, positive control and treatment control show in Fig. 10A. The result shows that HDFa cells was not dead-induced by DBDJ with plasma dissipated power at 0.5 watt and exposure time 1 min. Fig. 10B statistical analysis of cell viability percentage. The result has shown this condition has a high ability to eradicate bacterial and destroy bacterial biofilms without effect to the viability percentage of HDFa cells. The total apoptosis percentage of HDFa cells shows in Fig. 11. Accordingly, the apoptosis profile could distinguish 4 cell populations by this method: Live cells; early apoptotic cells; late apoptotic/dead cells; and necrotic cells. Fig. 11A presents DBDJ with plasma dissipated power at 0.5 W and exposure time at 1st min. increasing the total apoptosis percentage of HDFa cells slightly but this is not significant after used statistical analysis (see Fig. 11B). The result shows DBDJ plasma was not induced apoptosis and leading cell to death when compared with negative control and treatment control. In positive control was used DMEM +10% DMSO 2 hr for induced cells to apoptosis and dead cells.

The result from Live/Dead assay, Muse Count & Viability Assay and Muse Annexin V & Dead Cell Assay shown in the same direction is DBDJ without side effect to viability cell and not inducing apoptosis leading cell to death cell of HDFa cell shown in Fig. 9-11.

4. Summary

The DBDJ have a high bactericidal efficiency to S. aureus and P. aeruginosa at plasma dissipated power 0.41 to 0.50 watt and exposure time 45 to 60 s. The bacteria inhibition zone affected by RONS and charge particles. Electrostatic force of ions in plasma played an important role for bactericidal leading to cell lysis. The difference of cell wall structure bacteria leading to difference DBDJ sensitivity. DBDJ with plasma dissipated power at 0.5 watt and exposure time 1 min have high potential to killed bacteria, eliminate bacterial biofilms by without damage or side effect to cell viability, apoptosis and death of HDFa cells in vitro is essential for clinical use [5] which it have a high bactericidal efficiency and assist in wound healing.

Acknowledgements

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References


