Glow Discharge Plasma Sterilization of Prokaryotic Microorganism Through Electrostatic Plasma

Paola M. Morales Carvajal[1,4], Daniel Borromé[1,4] Ángel González, Ph.D[2,4], Wilfredo Fariñas, Ph.D[1,4]
[1]Biomedical Engineering Department, [2]Electrical Engineering Department, [3]Plasma Engineering Laboratory, [4]Polytechnic University of Puerto Rico

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Abstract

Sterilization/disinfection of target bacteria – *Bacillus Subtilis* – is investigated using plasma sterilization process which involves both **Dielectric Barrier Discharge Plasma (DBD)** and **Glow Discharge Plasma.** The purpose of this work is to identify the optimum parameters for sterilization of the target **microorganism** involving the use of **electrostatic plasma.** *B. Subtilis* is a diverse bacteria species found to be **highly resistant dormant endospores. [2]** Capable to growth in diverse environments – terrestrial and aquatics – including in the gastrointestinal tract of animals and humans. **[1], [2]**
The sterilization was carried out at **PUPR Plasma Engineering Laboratory** in which the voltage (460-500 Volts) and time intervals (15, 10, 5, 1 minutes) were varied. The system was pumped down to a vacuum level of 10^{-1} Torr during the sterilization process. **Flowing Plasma** was created in the chamber and interacted with the corning glass having direct contact with the bacteria. The flowing afterglow of the reduced pressure in ionized air provides photons over a broad ultraviolet wavelength range as the main biocidal agent of the *B. Subtilis*. **[1]** A 70% effectiveness of the bacteria sterilized in Glow Discharge Plasma was observed while The DBD was found non effective on the *Bacillus*.

Introduction/Background Information

Sterilization is a process that destroys or eliminates all forms of life and its classified in two methods: chemical and physical [1].
Chemical sterilization includes alcohols, halogens, aseptic techniques and gaseous method. **Chemical** sterilization causes complication because the microorganism will not be sterilized completely and can be hazardous. **Physical methods** are thermal heat treatment, radiation and filtration method. Therefore, an effective **sterilization** method can be conducted by **physical method sterilization as plasma. Plasma** is a matter that consists in ionized gas, positively charged ions, electrons and quasi-neutrals. **[3]** It is expected that **plasma** would sterilize the microorganism in a fast rate as compared to other sterilizations methods like heat and microwave treatment. **[1] Plasma Sterilization** is a technique that can achieve superior sterilization under chemical agents like gas. **[4]** As a result, the microorganism in plasma exposure will suffer the destruction of the DNA. **[1]** When such genomic structure is degraded it causes the erosion of the microorganism through the **spores - asexual reproduction characteristic**. In order to establish the parameters the variables that were recorded were the pressure, electrical current and voltage.

Problem Statement

According to **HAIs, (Healthcare Associate Infections)** a survey was carried out which reported **44.8%** of **415** people were involved with contaminated medical devices. In cases of **reused surgical equipment, 33%** are prone to transmit an infection. **[4], [5]**

Project Significance

Stimulate and **implement** sterilization using plasma for medical devices, reducing sterilization time and obtaining a medical device completely free of bacteria that could cause harm to the patient.

Project Objectives

Apply different types of plasma to determine the parameters at which the target bacteria is sterilized. Improving the current sterilization method.

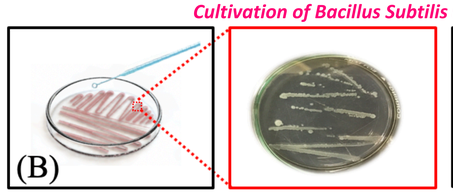
Project Scope

Develop a method of sterilization using plasma to establish sterilization parameters in surgical materials, to develop a sterilization method that can be rapid and effective.

Materials & Methods

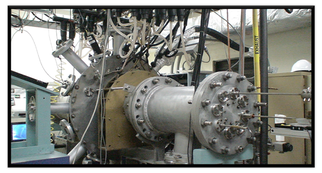
Cultivation of Bacillus Subtilis Petri Dishes

[Fig 1 (a)] Sterilization process in 45 minutes of the Corning Petri Dishes and Erlenmeyer flask (50 g) with 45g of Tryptic Soy Agar (TSA) in the Autoclave.



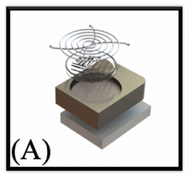
[Fig 1 (b)] Precautions were implemented depositing 7g of dehydrating medium in the Petri Dish corning. Then inoculation of *B. Subtilis* were performed using a 10μm needle loop in the biological cabin.

[Fig 1 (c)] *Bacillus Subtilis* inoculated Corning Petri dishes were incubated *in 34°C* for 24 hours for the bacteria growth.

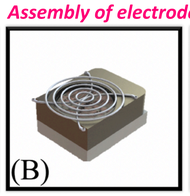


[Fig 2] Electron Cyclotron Resonance Plasma (ECRP) and Eletrostatic Plasma camera - Plasma laboratory at PUPR - machine were bacteria sterilization trials were placed.

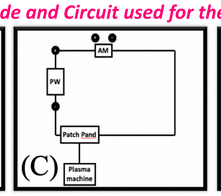
Assembly of electrode and Circuit used for the ECR Plasma Machine for DBD & Glow Discharge Plasma



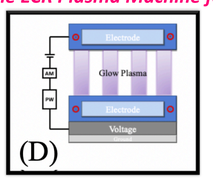
[Fig 3 (a)] Exploded view assembly with Teflon, aluminum base with applied voltage, petri dish and on top an electrode.



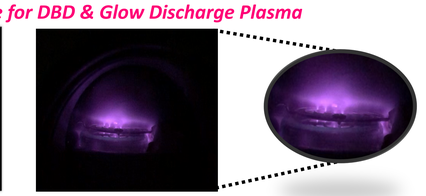
[Fig 3 (b)] Final product to locate in the Electrostatic plasma camera. (PUPR ECP machine)



[Fig 3 (c)] DC Circuit of the electrostatic plasma with an amperemeter, power supply connected to a patch band.

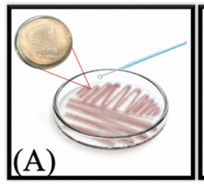


[Fig 3 (d)] Flowing Electrostatic glow plasma applied to the DBD and Glow Discharge plasma methods.

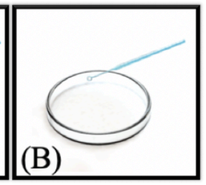


[Fig 3 (e)] Dielectric Barrier Discharge Plasma trial at PUPR Plasma Laboratory 05.15.2019. Showing direct glow through the Corning Petri dish.

Bacillus Subtilis post-Plasma Inoculation

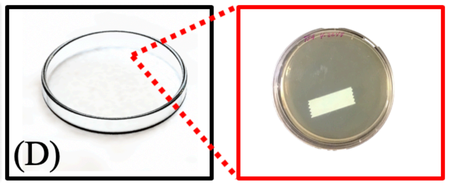


[Fig 4 (a)] After plasma exposure inoculating with 10μm needle loop process in plasma exposure Corning Petri dish (60 x 15 mm) is activated.



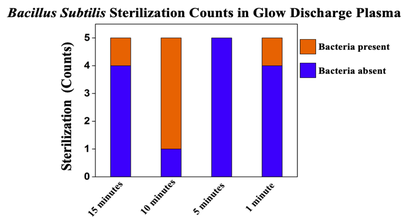
[Fig 4 (b)] Inoculated infected inoculating needle to the new cultures with 7g of TSA in a new Petri Dish corning.

[Fig 4 (c)] Inoculated new Corning Petri dishes were incubated *in 34°C* for 24 hours for the bacteria growth.

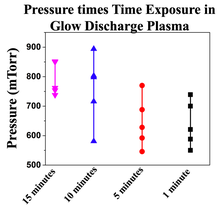


[Fig 4 (d)] After being incubated during a period of 24 hours, bacteria presence has to been seen if not, the plasma sterilization process was successful in Glow Discharge and DBD plasma.

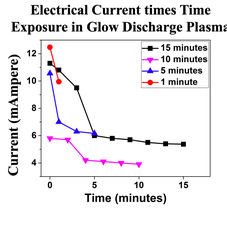
Sterilization Results and Data Analysis



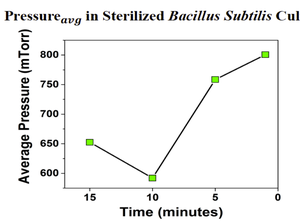
DATA 1 Sterilized bacteria in Glow Discharge plasma



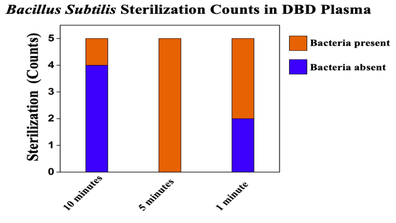
DATA 2 GDP for pressure measurements in time intervals



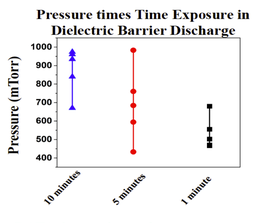
DATA 3 Electrical current for the GDP



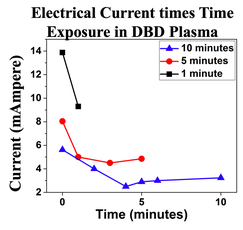
DATA 4 Average pressure of the sterilized B. Subtilis in the glow discharge plasma



DATA 5 Sterilized bacteria in Dielectric Barrier Discharge plasma



DATA 6 DBD plasma for pressure measurements



DATA 7 Electrical current for the DBD plasma

Discussion

According to the data obtained and the analysis of the graphs, sterilization by means of **Glow Discharge Plasma**, in which 20 sterilization trials were placed obtaining 70% of effectiveness and **Dielectric Barrier Discharge** method in which 15 exposure trials were carried out obtaining 30% of effectiveness. **[DATA (1, 5)].**
It is **speculated that not only the plasma ions influence the sterilization but also the electrical field that occurs at the time of exposure** plays a critical role. *Bacillus Subtilis* is **sterilized at a lower electrical current, higher pressure, and voltage greater than 460 Volts** in both methods**.** The most effective **Glow Discharge Plasma** process was in 5 minutes using a varied pressure of [550-750] mTorr. **[DATA (2)].**
From previous data obtained it is identified that for a successful sterilized bacteria with the Bacillus Subtilis properties its dissected that at a current range [7 – 11] mAmp and higher pressure of [550 – 750] mTorr its sterilized. **[DATA (2, 3)].** The 30% of DBD sterilization process was successful at a higher pressure [700-800] mTorr and a lower electrical current [2-6] mAmp using a voltage of 460 Volts.

Conclusion

Other methods of sterilization of surgical materials, for example the autoclave, involves a sterilization time of 45-60 minutes at 200°C. Compared to the type of plasma that was used to obtain positive results, it was sterilized in 5 minutes, voltage, a pressure and electrical current range mentioned in discussion using air as a component of gas to ionize. **Glow Discharge is more effective than sterilization of Dielectric Barrier Discharge.** Speculating an advance to the future development of establishing a methodology to sterilize surgical materials through plasma.

Future Work

This method would be further applied with other types of microorganisms of clinical interest. In which it can have the necessary data determining other variables, as the temperature of the electrons, the wavelength of UV radiation, the density and the physical properties of the bacteria after the sterilization process and to apply the process to surgical materials. In order **to develop the project on a large scale, designing and manufacturing a plasma sterilizer model** to be applied to hospital industries.

Acknowledgements

The authors would like to thank the Hispanic Serving Institution for support of this work through PUPR site Grant P031C160141 and HIS-STEM committee, the technical laboratory, faculty and directors of the Biomedical Engineering department, Prof. Joseph Morales, Plasma Laboratory technical, Daniel Borromé and Mathematics and Science Department Biology technical, Ermarie Hernandez, Program Education Service (PSE) faculty and the Mechanical Engineering department, Dr. Julio Noriega , for the equipment's and support.

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