Sterilization and Inhibition of Bacteria Growth by Polymer Film Barriers Deposited Using a Floating-Electrode Dielectric Barrier Discharge Plasma Jet in Ambient Environment Conditions

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Abstract: Inhibition of bacteria growth on agar plates was achieved by the polymer films deposited using a floating-electrode dielectric barrier discharge jet (DBD jet). The DBD jet, which has been known to have sterilization properties, was employed as a polymer depositing tool in this study. A mixture of helium (He) and a small amount of methyl methacrylate (MMA) was used as the plasma working gas for the deposition of Poly(methyl methacrylate) (PMMA) films. Escherichia coli and Burkholderia glumae (a seedborne pathogen to cause bacterial panicle blight on rice) were employed for the study of bactericidal activity and bacterial growth inhibition. The DBD jet fed by He/MMA was observed to create a larger sterilized region than the DBD jet with pure He. Based on the observed density of bacteria colonies, the growth of the bacteria was seen to be significantly inhibited on the areas where PMMA covers the agar. It was also observed that the bacterial growth inhibition was influenced by the thickness of deposited films. This study shows that the ambient DBD-jet-based polymer deposition technique provides a new way of medical wound treatment; that is, it can not only sterilize the wound but also create a polymeric ‘bandage’ on the wound surface to prevent bacteria from causing any further infections.

Keywords: Dielectric barrier discharge (DBD), film deposition, plasma jet, bactericidal activity

1. Introduction

Atmospheric pressure non-thermal plasmas have received attention in recent years owing to their potential in medical applications. Such uses include bactericidal activity [1], protein destruction [2], cell inactivation [3] and blood coagulation [4]. In terms of wound treatment, conventional approaches employed laser or thermal surgery, which causes cell necrosis and permanent damage of tissue due to extreme temperature [3]. Instead, it has been demonstrated that selective antiseptic activities without damaging surrounding tissue can be accomplished by using non-thermal plasma-based devices. In vivo treatment with the ability to detach localized cells with minimum cell destruction has also been shown [5]. Thus non-thermal plasma methods have been considered as a promising substitute for the conventional thermal treatment of wounds.

To achieve localized treatment on wounds with non-flat surfaces, the handheld atmospheric pressure non-flat surfaces, the handheld atmospheric pressure dielectric barrier discharge jet (DBD jet) with floating-electrode [6] is particularly amenable for medical applications. The floating-electrode DBD jet employs only one electrode (powered electrode), and the downstream substrate is used as the second electrode (grounded or at a floating potential). It is a direct plasma method, which allows for more species in the vicinity of the substrate, compared to those indirect plasma methods [1]. Due to its efficient reaction chemistry, high plasma stability, and low power consumption, the floating-electrode DBD jet was also employed to modify material surfaces and polymer treatment besides sterilization. Recently we have reported on the use of floating-electrode DBD
jet as a depositing tool for polymers on temperature sensitive substrates [7]. Considering that film deposition can be achieved by the DBD jet, on infected wounds not only can the surface be sterilized but also protected from the future infection, as illustrated in Figure 1.

In this paper, polymer film deposition on agar plates was performed using the floating-electrode DBD jet. The agar acts as a simplified, temperature sensitive, moist, nutrient rich surrogate for an actual wound. The jet’s sterilization ability was reported and compared with a conventional DBD jet. Inhibition of bacteria growth on the deposited films was investigated. The dependence of the efficacy of bacterial growth inhibition on film thickness was also presented.

2. Experimental Apparatus & Methods

Figure 2 shows the experimental setup for polymer deposition on the agar surface in a petri dish using a floating-electrode DBD jet and more detail can be found in [7]. Industrial grade helium (He 99.995%) was utilized as the working gas for plasma generation. Methyl methacrylate (MMA) liquid monomer (Sigma-Aldrich, 99%) was employed as the polymer precursor and vaporized by feeding He through a glass bubbler at room temperature. The working gas flow rate was set to 2.9 slm (standard liter per minute), while the flow rate of the MMA carrier gas was 0.1 slm. The mixture of the working gas and the carrier gas was then directed through a DBD jet generator. A petri dish filled with agar was placed on an aluminum holder downstream of the jet for plasma treatment and poly(methyl methacrylate) (PMMA) film deposition. A high voltage with sinusoidal waveform at 28.5 kHz was applied to the powered electrode. A discharge power of about 1.2 W was employed in this study.

Two bacterial strains, Escherichia coli (E. coli) and Burkholderia glumae (B. glumae, a seedborne pathogen to cause bacterial panicle blight on rice), were prepared in liquid media with a concentration of 10^9 colony-forming units per mL (CFU/mL) and transferred onto the agar plates before treatment. The illustrations for examination of the bactericidal activity and bacterial growth inhibition can be seen in Figure 3. In the sterilization test, the He/MMA DBD jet was used to treat the agar plate immediately after the bacteria media were plated on the agar [Figure 3(a)]. Since the DBD jet has been known to have the sterilization properties, after the dishes were placed in an incubator for one day the growth of bacteria colonies can be observed at those untreated areas, as shown in Figure 3(b). Note that the term sterilization here is used to refer to the lack of reproduction and colony growth by the bacteria. Using He/MMA DBD jet a PMMA film was obtained on agar. For observation of bacterial growth inhibition by the polymer film, additional bacterium suspensions were dropped on the treated area [Figure 3(c)]. The polymer film barrier would lead to no observation of bacteria colonies on the area owing to the fact that the film would prevent the bacteria from reaching the nutrient agar [Figure 3(d)]. For comparison, a DBD jet fed by pure He at 3.0 slm (He DBD jet) was also used as an experimental control.

3. Results & Discussions

Figure 4(a) shows the image during film deposition on agar by the DBD jet. A thin transparent PMMA film was grown on the agar. Note that long treatment duration may lead to the creation of a cavity on the
agar due to gas flow and heating effect of the DBD jet. This can be seen from the result after 10 min deposition shown in Figure 4(b). In addition to the transparent film an opaque film was observed at the region directly under the center of the plasma jet. The opaque film was formed likely due to ‘buckling effect’ [8] caused by relatively high deposition rate of PMMA. A more detailed explanation of the opaque film was also reported in our earlier work [7].

For clear visualization of the as-deposited PMMA film a modified lift-off technique was used wherein a 25-mm square of the agar was cut from the treated region and placed in near-boiling water. The hot water resulted in the dissolution of the agar, leaving behind the PMMA film, as displayed in Figure 4(c). Furthermore, the film was taken out of the water and placed on a microscope slide. This method was utilized to obtain the films with different deposition durations (1, 5 and 10 min), as presented in Figure 4(d). It can be seen that in the cases of 5 and 10 min the films appeared to have dimensions greater than 25 mm. A distinct circular pattern was observed in the middle in each of these two cases probably due to the growth of a relatively thick film in the region directly downstream of the DBD jet. The circular pattern in the 10 min case was seen to have larger dimension (11 mm in diameter) than the one (8 mm in diameter) after 5 min deposition. It should be mentioned that the deposited film appears thinner towards the outer region. In the 1 min case only a small piece of film was observed on the microscope slide likely due to the fact that the outer part of the film was too thin to remain intact during the lift-off process. These results indicate that a longer treatment time leads to a thicker deposited film covering a broader area.

For the tests of sterilization properties, 1 min treatment of the He/MMA DBD jet on agar was performed immediately after the bacterium suspensions were transferred to the agar surface. He gas, He/MMA gas and He DBD jet were also used as experimental control. Figure 5 shows the images of the treated petri dishes after one-day incubation. No sterilization was observed at the areas treated by the He gas and the He/MMA gas, as shown in Figure 5(a) and 5(b), respectively. The dashed circles on the images indicate the inner dimension of the tube (3.96 mm in the inner diameter). Using He DBD jet, the sterilized area was seen to have similar dimension to the tube [Figure 5(c)]. However, it was observed that in Figure 5(d) the He/MMA DBD jet created a greater sterilized area (around 18 mm in the diameter) than that by the He DBD jet. The relatively large sterilized area implies that either 1) active species generated from MMA in the DBD aid in the sterilization or 2) deposition may result in a film with a diameter of at least 18 mm and this film inhibits the growth of bacteria under the film.

Bacterium suspensions were then dropped on the
Figure 6. Comparison between the treated areas without (leftmost) and with drops of bacterium suspensions at various plasma treatment durations in different cases: (a) *E. coli* treated by He DBD jet; (b) *E. coli* treated by He/MMA DBD jet; (c) *B. glumae* treated by He DBD jet; (d) *B. glumae* treated by He/MMA DBD jet.

treated regions for observation of inhibition of the bacteria growth by the deposited PMMA films. The right three columns in Figure 6 show the treated areas with the drops of the bacterium suspensions after one-day incubation, while the images in the leftmost column are the cases without the additional bacterium drops for comparison. Again, three different deposition times (1, 5, and 10 min) were employed in the case using He/MMA DBD jet [Figure 6(b) and 6(d)] for studying the effect of the film thickness. Figure 6(a) and 6(b) are the *E. coli* plates treated by the He DBD jet and the He/MMA DBD jet, respectively. It can be observed that the colonies of *E. coli* still grew on both the areas treated for 1 min. However, when the deposition time was extended to 5 min the *E. coli* growth was observed to be inhibited at the treated area. 10 min deposition time showed a similar result. In the case of *B. glumae* treated by the He DBD jet [Figure 6(c)], the densely grown *B. glumae* colonies still formed on the agar, while no growth of *B. glumae* colonies was observed at the area treated by the He/MMA DBD jet for deposition time of 1 min and greater, as shown in Figure 6(d).

These results show that the polymer film barriers deposited by the He/MMA DBD jet can inhibit the bacteria growth. The efficacy of the bacterial growth inhibition was influenced by the thickness of deposited films. Different strains of bacteria showed different sensitivity to the environment they grow on.

4. Conclusion

In this paper we reported that PMMA films can be deposited on agar using a floating-electrode DBD jet fed by a mixture of He and MMA. A greater sterilized region on the bacteria culture plate was created by the He/MMA DBD jet, compared to that treated by the pure He DBD jet. The enlarged sterilized area was probably attributed to active species from MMA or the deposits themselves. It was observed that the bacteria growth on the deposited PMMA films was significantly inhibited, and the inhibition efficacy showed dependence on the film thickness. With this new DBD-jet application, wounds can be not only sterilized but also protected by the deposited coatings from bacteria growth.

References