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Original paper

Direct-Contact and Contact-free Treatment of a DBD-like 1on-thermal Atmospheric Pressure Plasma Jet on *Pseudomonas aeruginosa*

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Abstract In this study, a Dielectric Barrier Discharge (DBD)-like non-thermal atmospheric pressure plasma jet (NTAPPJ) was exposed to the *Pseudomonas aeruginosa* planktonic bacteria. The P. aeruginosa bacteria were treated by the NTAPPJs in both Ar and He at the non-contact distance (contact-free) and the contact distance (direct-contact). The contact of the NTAPPJs with the bacteria has generated spark discharges in both filamentary mode and diffuse mode. The antibacterial efficacy of the Ar and He NTAPPJs for various treatment times $(60, 120, 180,$ and 300 sec) were investigated by treatment on the bacteria without contact and with contact in both filamentary mode and diffuse mode. The NTAPPJs in both Ar and He caused erosion on the agar surface when treated in direct-contact with the bacteria in the filamentary mode, while the NTAPPJs in Ar in the diffuse mode produced the greatest antibacterial efficacy without damage.

Keywords

DBD-like jets, Non-thermal Atmospheric Pressure Plasma Jet, Direct and Indirect treatment, Planktonic Bacteria, Pseudomonas aeruginosa.

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Introduction

Plasma is a partially or fully ionized gas consisting of various particles, such as electrons, positive/negative ions, neutral atoms/molecules (can be radicals), excited atoms/ molecules (can be metastables), and photons (UV, visible, and thermal radiation). Plasmas are generally divided into thermal and non-thermal in terms of comparing the temperatures of the electrons with the temperatures of the other particles in plasma. While the temperatures (i.e. kinetic energy) of the particles in the plasma are equal in thermal plasmas, the temperatures of the electrons in non-thermal plasmas are higher than the temperature of the other particles, and the gas temperature remains as low as room temperature (Raizer, 1991; Grill, 1994). Non-thermal plasmas have many advantages such as the effects of low temperature, low electric field, and the chemical interactions of the active radicals have various applications. In the last decades, non-thermal atmospheric pressure plasma sources, such as corona, streamer, dielectric barrier discharge (DBD), surface dielectric barrier discharge (SDBD), and plasma jet, have attracted significant interest, because they do not require expensive vacuum equipment and can be readily applied (Lu et al., 2016; Domonkos et al., 2021; Ilik et al., 2020).

Non-thermal Atmospheric Pressure Plasma jets (NTAP-PJs) also belong to the large family of non-thermal atmospheric pressure plasmas; they are discharged plasma that can extend outside the plasma generation region between electrodes into the surrounding ambient environments. NTAPPJs are playing an increasingly important role in various plasma processing applications. This is because of their practical capability to provide plasmas that are not spatially bound or confined by electrodes (Laroussi and Akan, 2007; Penkov et al., 2015). Moreover, they can be movable and easily applied to samples with inner surfaces. NTAPPJs generated in air consist of different components such as neutral atoms/molecules, radicals, excited atom/molecules, metastables, charged particles (ions and electrons), reactive oxygen species (ROS), reactive nitrogen species (RNS), photons of UV, visible, infrared, and thermal radiation, and electromagnetic field, all of which may contribute to its antibacterial properties (Khlyustova et al., 2019).

Among other applications, the treatment of temperaturesensitive surfaces such as biological material is of interest, in particular in the field of plasma medicine by the interaction of plasma with living cells, tissues, and bacteria, e.g., for inactivation or sterilization of bacteria, fungi, and spores, cancer treatment, wound healing, dentistry, genetics, and skin treatment (Wu et al., 2016; Lin et al. 2016; Mashayekh et al., 2015; Schmidt et al., 2017; Yaopromsiri et al., 2015; Laroussi, 2015). The temperature-sensitive surface treatment of NTAPPJs has also produced amendatory results in the fields of food safety (Pankaj et al., 2018), agriculture (Velichko et al., 2019), and textile (Peran and Ercegović, 2020).

Biological materials can be treated by plasma in two different methods (Akan and Çabuk, 2014; Saadati et al., 2018 ; Malyavko et al., 2020; Dobrynin et al., 2009; Dobrynin et al., 2011; Morris et al., 2009): Direct treatment is when the biological sample to be treated is in direct contact with the plasma. All plasma agents (plasma-generated species and electromagnetic fields) come in contact with the sample. The most important distinguishing feature of the direct plasma treatment is that a significant flux of charges (ions and electrons) reaches the surface of the sample. The second method is the indirect treatment in which the biological sample is placed at a non-contact distance from the plasma. In this treatment, the charged particles do not play a role since they recombine before reaching the sample, and the active agents are blown to the treated sample. In indirect treatment, the active uncharged species are typically delivered to the surface via a flow of gas through a plasma region. Although mostly active uncharged species (e.g., O, O_2 , OH, NO, O_3) will be the acting agents , many of the short-lived neutral reactive oxygen or nitrogen species also do not reach the sample. To date, direct and indirect treatment of bacteria using nonthermal atmospheric pressure plasma in the air is compared in terms of bacterial inactivation rates. It is demonstrated that direct treatment, where charged particles contact with bacteria directly , produces inactivation much faster than the indirect treatment, where plasma afterglow (post-discharge) is delivered to the bacteria with a gas flow through the plasma region (Dobrynin et al., 2011; Morris et al., 2009).

NTAPPJs consist of a gas tube equipped with one or two electrodes. The plasma is ignited inside the tube and transported to the outside as well as to the sample to be treated by a gas flow. NTAPPJs can be distinguished between remote plasmas, where the plasma is potential free and consists of recombining active species, and active plasma jets where the expanding plasma contains free and high energetic electrons. In the latter case, the targeted sample forms a second or third electrode. In many cases, the voltage does not need to be directly connected to this sample electrode, but some current may flow through the sample in the form of either a small conduction current, displacement current, or both. NTAPPJs can be classified into four categories, i.e., dielectric-free electrode (DFE) jets, dielectric barrier discharge (DBD) jets, DBD-like jets, and single electrode (SE) jets (Lu et al., 2012). When the plasma jet is not in contact with any sample (cells or whole tissue), the discharge is like a DBD. However, when the plasma jet is in contact with a

sample, the discharge is running between the high voltage electrode and the sample to be treated. In such a case, it no longer operates as a DBD will work as a streamer, spark, or arc discharge. The NTAPPJ used in this study is a DBD-like jet with and without contact with P. aeruginosa planktonic bacteria. Although NTAPPJs are accepted as remote or indirect plasma applications on a sample, DBD-like type jets should be classified according to whether they are in contact with the treated sample. DBD-like type jets change the jet format and transit to spark or arc discharge when contacted with the treated sample. In this case, they are separated from the remote application method by transferring charged particles to the sample to be treated. In addition, when a DBDlike type jet is in contact with a sample, it may create an undesirable chemical or physical effect on the sample to be treated. In this study, the effects of a DBD-like type jet exposed to P. aeruginosa planktonic bacteria with and without contact were investigated.

Experimental

Non-thermal Atmospheric Pressure Plasma Jet (NTAPPJ) System

For the non-thermal plasma treatment of P. aeruginosa planktonic bacteria, a homemade non-thermal atmospheric pressure plasma jet (NTAPPJ) device was used. A schematic of the NTAPPJ device used, including the spectroscopic measurement system, is given in Fig. 1. In this work, the NTAPPJ device was attached to a support with the jet (plume) shooting downward. Petri dishes containing P. aeruginosa planktonic bacteria evenly spread over agar were put right under the plasma jet and on a platform the height of which can be adjusted. In this way, the distance between the nozzle (end of the quartz tube) of the NTAPPJ and the Petri dishes containing P. aeruginosa planktonic bacteria to be treated, which is called the exposure distance (ED), can be determined.

The NTAPPJ source consists of two concentric electrodes through which a gas flow. By applying kHz power to the inner electrode, the gas discharge is ignited. The ionized gas from the plasma jet exits through a quartz glass tube, where it is directed onto a substrate (in this study P. aeruginosa planktonic bacteria) a few centimeters downstream. A device that is very similar to the NTAPPJ device used in this study was investigated in our previous article (Ilik and Akan 2016). The NTAPP jet assembly consisted of a 100 mm-long quartz tube with a gas inlet, and the inner and outer diameters of the quartz tube are 4 and 6 mm, respectively. A coaxial dielectric barrier configuration was used for the electrode geometry. A tungsten needle with a diameter of 0.5 mm and length of 150 mm is inserted into the center of the quartz tube as a high-voltage electrode. The high-voltage electrode is fixed at a distance of 3 mm away from the open end (the nozzle) and it was insulated with rectangular cubical polyethylene material. A 0.70 mm-thick copper foil with a 10 mm-width was used as the ground electrode and was wrapped on the quartz tube with the outer edge of the electrode at the downstream side being 20 mm away from the orifice of the quartz tube.

High-purity He (99.999%) and Ar (99.999%) enter the tube from the top side of the quartz tube, which is controlled by a mass flow meter division in a range of $0-15$ L/min.

Figure 1. Schematic of the NTAPP jet device used.

A homemade power supply was developed to generate the plasma using a sinusoidal high voltage. For all the inactivation experiments reported in this letter, the peak-to-peak voltage of a 15 kHz sinusoidal waveform was optimized and then fixed at 18 kV.

In the NTAPPJ, the electron collisions excite the plasma species to the higher atomic or molecular levels decaying and emitting a photon at the specific wavelengths. These processes can be detected and analyzed by recording the emission spectrum. Optical Emission Spectroscopy (OES) has been applied to identify the chemical species present in the NTAPP jet (plume) when Ar and He is used as a working gas (Fig. 1). The emission spectrum for the Ar and He NTAPP jets in the air was taken by an Ocean Optics minispectrometer USB 2000+XR1-ES equipped with a 2048element linear silicon charge-coupled device (CCD) array detector. The 300 grooves/mm grating and 10-mm wide slit were used to evaluate spectra in the spectral resolution of the spectrometer 0.1 nm.

Bacterial Strain and Growth Condition

The standard bacterial strain was stored in a deep freezer (Eskisehir Osmangazi University, Biotechnology Laboratory) at -80 \degree C. The bacterial strain was inoculated into Tryptic Soy Broth (TSB) and incubated aerobically at 37°C for 24 hours. The strain was then subcultured on Tryptic Sov Agar (TSA) under the same conditions. To determine the region of growth inhibition, an overnight bacterial culture was diluted ($1/1000$) in sterile 0.9% saline, 100 µL of the diluted bacterial suspension was then spread evenly on the surface of the TSA plate. It was then immediately treated with the NTAPP jets at specified times. After exposure to the plasma jet, the plates were incubated for 24 hours at 37°C in a static incubator, and photographs of the agar plates showing the bacterial growth inhibition zones were taken using a digital camera. For control experiments, samples are treated by working gas flowing at the same flow rate with the plasma turned off. It should be noted that all experiments reported in this letter were repeated three times and the results were consistent with the same experimental conditions.

Results and Discussion

Non-thermal Atmospheric Pressure Plasma Jet (NTAPPJ) Generation

The origin of non-equilibrium plasmas is based on the development of electron avalanches. The main ionization mechanism in most gases is direct electron impact ionization by free electrons accelerated by the electric voltage applied. At low pressure, the positive ions generated in the ionization process drift to the cathode and lead to secondary electrons emission. This following delivery of free electrons seeds new avalanches, and this is the main characteristic of the Townsend-breakdown mechanism that leads to the generation of a self-sustained gas discharge such as a glow discharge. At higher pressure, the number of ionizing collisions per unit volume increases, and the breakdown mechanism changes significantly. When electron avalanches generate a space charge that locally enhances the electromagnetic field applied, secondary electron avalanches are started in the gas phase. The ionized region and its perturbation of the electromagnetic field grow rapidly and eventually form a separate plasma channel (a streamer-breakdown mechanism) called a streamer. A streamer propagates in the discharge region but does not necessarily connect the two opposite electrodes. Streamer discharges can undergo a transition to more intense spark discharges when it reaches to opposite electrode. An intense spark channel can transit into an arc discharge if the power supply can deliver the necessary current (Bruggeman et al., 2017).

When He is introduced to the NTAPPJ system given in Fig. 1 with a gas flow rate of 5 L/min, the plasma jet is formed in the filamentary mode as seen in Fig. 2a. When the gas flow rate of He is increased to 10 L/min, the length of the plasma jet increases as seen in Fig. 2b, and the plasma jet appears more in diffusive mode. The length, shape, and mode of the plasma jet in He were influenced by the gas flow rate. In filamentary plasmas, in contrast to the mostly diffusive plasmas, the activity of atmospheric pressure plasmas is strictly localized. The formation mechanisms of the diffusive and filamentary of NTAPPJs have been extensively studied (Li et al., 2017). When the NTAPP jet generated in the diffusive mode using He with a flow rate of 10 L/min is exposed to the agar in the petri dish containing *P. aeruginosa* planktonic bacteria, the jet transits to the spark discharge mode as seen in Fig. 2c. For the direct-contact treatment, the bacterial samples on the agar plates are placed right under the plasma jet at an adjustable distance from the nozzle. The bacterial samples are directly contacted with the plasma jet (plume). In this case, many streamers are formed in the area where the plasma jet contacts the agar, and these streamers move around the contact area without causing any visible damage. The agar temperature measured with the standard Ktype thermocouple connected to a digital multimeter (Fluke 179) and IR thermometer (Benetech GM320) almost does not change and remains around 25° C during the treatment of 5 min. When the NTAPP jet generated in the filamentary mode using He with a flow rate of 5 L/min is exposed to the agar, the jet is formed in the spark discharge mode as seen in Fig. 2d. In this case, streamers are not formed, and the jet contacts a point on the agar producing a spot (like a small

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point). This spot may create a physical or chemical erosion on the agar. After 5 minutes of treatment, the agar temperature increased up to 27°C.

Figure 2. Plasma modes that occur at different gas flow rates and direct-contact treatment on the P. aeruginosa planktonic bacteria with the NTAPPJ system.

As can be seen in Fig. 2e and Fig. 2f, the NTAPP jet in Ar with a flow of 5 L/min, which was formed in the filamentary mode, transits to the diffusive mode by shortening when the Ar flow rate was increased to 10 L/min. When the NTAPP jet generated in diffusive mode using Ar with a flow rate of 10 L/ min is exposed to the agar, the jet transits to the spark discharge mode as seen in Fig. 2g. When the NTAPP jet generated in the filamentary mode using Ar with a flow rate of 5 L/min is exposed to the agar, the jet is formed in the spark discharge mode as seen in Fig. 2h. No chemical or physical effect was observed on the agar treated by the spark discharge in the diffuse mode of the NTAPP jet in Ar, while a spot was formed when the agar is treated by the filamentary mode. When the agar plates were

treated by both modes of the NTAPP jet in Argon, the temperature of the agar has changed between 25-27 °C.

Antibacterial Efficacy of Direct-Contact and Contactfree Treatment of the NTAPPJ on P. aeruginosa

The P. aeruginosa bacterial samples are treated by the NTAPPJ in two different ways, i.e., direct-contact and contact-free treatments. For the direct-contact treatment, the bacterial samples on the agar plates were placed right under the plasma jet at an adjustable distance from the nozzle. The bacterial samples were directly contacted with the plasma jet to generate a spark discharge. The first group of the experiment is done with Ar in the diffusive mode with a flow rate of 10 L/min and the filamentary mode with a flow rate of 5 L/min, respectively. For the control experiment, the bacterial samples are treated by the Ar at the same flow rate with the plasma off (power off).

Control plates (no plasma and Ar gas exposure, and Ar gas exposure without plasma ignition) exhibited consistent bacterial lawns, whereas the NTAPPJ-treated plates of the P. aeruginosa bacterial strains were characterized by the presence of clear zones (killing region) of growth inhibition (Fig. 3). The yellow arrows show the bacteria growth inhibited region after the plasma treatment. The bacteria-containing agar plates were placed under the NTAPPJ in Argon for the following time intervals: 60, 120,180, and 300 seconds. After incubation, photographs of the agar plates, showing bacterial growth inhibition zones, were taken using a digital camera. The Ar-NTAPP jet-exposed plates showed significant bacterial inhibition zones which indicate the extent of bactericidal activity of the plasma jet against P. aeruginosa. Although the maximum diameter of the plasma jet (plume) observed in the NTAPPJ in Ar is less than 2-3 mm in the filamentary mode and 4-6 mm in the diffusive mode, inhibition zones of several centimeters are obtained; which indicates that plasma-derived active species are not confined within the visible plasma plume, rather they are present in a larger volume around it. The observable inhibition zone diameter increased with increasing plasma exposure time as can be seen in Fig. 3. The inhibition zone diameter of the filamentary mode-NTAPPJ direct-contact treatment is smaller than that of the diffusive mode-NTAPPJ direct-contact treatment when Ar is used as a working gas. The damage on the agar plates by heating or etching was not observed in the diffusive mode-NTAPPJ direct-contact treatment (Fig. 3d), but spots were observed in the filamentary mode treatment (Fig. 3e). As mentioned earlier, the bacteria are affected by charged particles and reactive species simultaneously in direct-contact treatment.

When all the processes in obtaining the results in Fig. 3, when Ar is used as working gas, were repeated for He, the

Figure 3. Results of antibacterial direct-contact treatment of P. aeruginosa on the agar using the different Ar-NTAPPJ working modes as well as different treatment times. a.) Control plate (no gas and plasma exposure), b.) Control plate (Argon gas exposure without plasma ignition), c.) and d.) the variation of observable inhibition zone diameter with the Ar diffusive and filamentary mode-plasma exposure times of 60, 120, 180, and 300 sec., respectively. ED was 20 mm.

results given in Fig. 4 were obtained. When He is used as a working gas, the inhibition zone diameter in the diffusive mode was larger than that of the filamentary mode of the NTAPPJ in direct-contact treatment.

Comparing the antibacterial efficacy of the NTAPP jets in Ar and He after direct-contact with the bacteria, the direct-contact treatment of the Ar-NTAPP jet on the bacteria showed a more effective antibacterial efficacy than that of the He-NTAPPI

For the contact-free treatment, the P , aeruginosa bacterial samples on the agar plates were placed right under the plasma jet at a distance where they will not contact with the jet, that is, no spark discharge will occur. When the Ar and He NTAPP jets were exposed to the bacteria without contact in the diffuse mode, they produced a zone diameter, while no significant antibacterial effect was observed in the filamentary mode. When the Ar-NTAPPJ in the diffusive mode was exposed to the bacteria without contact, a larger inhibition zone was observed than when the He-NTAPPJ in the diffusive mode was exposed to the bacteria without contact as can be seen in Fig. 5.

When the P. aeruginosa are treated by the NTAPP jet in direct-contact using Ar and He, they are exposed simultaneously to charged particles (electrons and ions), UV/visible light, reactive oxygen species (ROS) such as ozone (O_2) , atomic oxygen (O), electronically excited oxygen (O, $({}^{1}\Delta)$), superoxide (O_2^-) , hydrogen peroxide (H_2O_2) , hydroxyl radical (OH) and anion (OH⁻), reactive nitrogen species (RNS) such as atomic nitrogen (N), electronically excited nitrogen $(N₂(A))$, nitric oxide (NO), and other excited molecular and atomic species of working gas, all of which may contribute to its antibacterial properties (Nicol et al., 2020; Brun et al., 2018), and thus maximum inactivation effect is obtained in this case. As the plasma jet is launched in the surround-

Figure 4. Results of antibacterial direct-contact treatment of P. aeruginosa on the agar using the different He-NTAPPJ working modes as well as different treatment times. a.) Control plate (no gas and plasma exposure), b.) Control plate (Helium gas exposure without plasma ignition), c.) and d.) the variation of observable inhibition zone diameter with the He diffusive and filamentary mode-plasma exposure times of 60, 120, 180, and 300 sec., respectively. ED was 20 mm.

Figure 5. Results of antibacterial contact-free treatment of *P. aeruginosa* on the agar using the Ar and He NTAPPJs at the different treatment times. **a.**) Control plates (no gas and plasma exposure), **b.**) Control plates (Argon and Helium gas exposure without plasma ignition), c.) and d.) the variation of observable inhibition zone diameter with the Ar and He contact-free NTAPPJ in the diffusive mode exposure times of 60, 120, 180, and 300 sec., respectively. ED was 60 mm.

ing room air, oxygen and nitrogen-based reactive species are produced even if the plasma jet operating gas itself does not contain oxygen or nitrogen. Hydroxyl (OH) is mainly produced by water molecules when struck by high-energy photons and high-speed electrons or molecules. The obtained results of the contact-free NTAPP jet treatment in both Ar and He show that it is less effective, probably due to the absence of charged species in the plasma jet. Bombardment on the cell wall by charged particles, electrons, and ions (positive ions are generally N_2^+ , while negative ions are O_2^-) can break chemical bonds, cause erosion through etching, formation of lesions and openings in the membranes, inducing further penetration of plasma toxic compounds inside a bacterial cell (Dobrynin et al., 2009; Bourke et al., 2017)

Fig. 6a and b show typical emission spectrums for the Ar and He NTAPP jet in the air, taken by an Ocean Optics mini-spectrometer USB 2000+XR1-ES as can be shown in Fig. 1.

We can compare the emission spectra between the He and Ar NTAPPJs given in Fig. 6a and b. The emissions of nitrogen second positive band (N_2) , atomic oxygen band (O) , and OH band were observed in both plasmas, while the emissions of nitric oxide (NO) and the first negative band of nitrogen molecule ions (N_2^+) were observed only in the He-NTAP plasma jet. This is due to the ionization potential of nitrogen molecules, which is 15.5 eV. The metastable levels of Ar are 11.5 and 11.7 eV; the energy provided from Ar metastable atoms is not adequate for the ionization of nitrogen molecules (Shao et al., 2012; Shao et al., 2015). In pure He and Ar, the intensity of O line (777 nm) in the Ar-NTAP plasma jet was slightly smaller than that in the He-NTAP plasma jet, whereas the intensity of OH line in the Ar-NTAP plasma jet was extremely higher than that in the He-NTAP plasma jet. The ionization energy of He is 24.6 eV, and the energies of two kinds of He metastable formed in gas discharge are 19.82 eV and 20.06 eV. Besides, as can

Figure 6. Emission Spectrums of the NTAPP jet (plume) in **a.)** Argon, and **b.)** Helium.

electrons and more suitable for inactivation. The results show that the antibacterial efficacy of the Ar-NTAP plasma jet is much better than that of the He-NTAP plasma jet. Because of the lower ionization energy (15.8 eV) of Ar, there are more ions and electrons in the Ar-NTAP plasma jet than that in the He (ionization energy is 24.6 eV) NTAP plasma jet, which will lead to a bigger discharge current. This might be an explanation why the Ar direct-contact plasma jet treatment on the *P. aeruginosa* is more efficient than the He direct-contact NTAP plasma jet treatment.

He-NTAP plasma jet, which was related to its high-density

From the results, it is expected that OH and charges are the most important species for inactivation in treatment between He and Ar NTAP plasma jets. The main agent which induces the difference of antibacterial efficacies between He and Ar NTAP plasma jets is the OH radical and charge activity. The generation of ozone (O_3) can also be checked by the unique smell of ozone in the He and Ar NTAP plasma jets. The Ar-NTAP plasma jet came out to have an intense fishy smell of ozone compared with the He-NTAP plasma jet. Because the lifetime of ozone is longer than other radicals (Jablonowski et al., 2018), ozone might play a crucial role in the inactivation process in the Ar-NTAP plasma jet.

Conclusions

The DBD-like NTAP plasma jet system used in this paper generates the filamentary mode jet at low gas flow rates and the diffuse mode jet at high gas flow rates. The NTAP plasma jets in Ar and He generated in both modes create a spark discharge when they are in contact with the agar containing the *P. aeruginosa* bacteria. While the spark discharges in contact with the agar in filamentary mode cause a visible erosion on the agar, the spark discharges in contact with the agar in diffusive mode do not cause any erosion.

The antibacterial efficacy of the Ar and He NTAPPJs for various treatment times $(60, 120, 180,$ and 300 sec.) were investigated by treatment on the *P. aeruginosa* bacteria without contact and with contact in both filamentary mode and diffuse mode. We compare the effectiveness of direct-contact and contact-free antibacterial treatment by the NTAPPJ generated using the same discharge setup using He and Ar and demonstrate that the direct-contact treatment can achieve antibacterial efficacy much better without any physical or

Direct-Contact and Contact-free Treatment chemical erosion. The *P. aeruginosa* bacteria treatment by direct-contact with the Ar-NTAPPJ showed more antibacte-

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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