Preliminary Investigations of Optical Emission Spectroscopy Atmospheric Plasma for Microbial Inactivation

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***Abstract*—This paper explores the reactive species in helium plasma based on study of microbial inactivation effect. Optical emission spectroscopy was employ to analyze basic mechanism of plasma for microbial inactivation. Inactivation process was carried on bacteria Escherichia coli. Bacteria were fully inactivated at helium flow rate 600 ml/min. There are variations of each reactive species in different flow rate such as volume of species and intensity. The reactive species such as nitrogen, oxygen, helium were detected using spectrometer. Every species in plasma flume play important role in inactivation process. By observing the emission spectrum, it enables to understand and optimizing the microbial inactivation process and also gives insight in the plasma physics and chemical process.**

***Keywords* - Escherichia coli, microbial inactivation, optical emission spectroscopy, atmospheric plasma jet, sterilization**

I. INTRODUCTION

Cold atmospheric plasma is well known for its application as biocides devices for disinfection purpose in health and hospital care [1]. In the medical field, pathogenic bacteria and sterilization medical apparatus is one of important issues that has been the focus by investigators and researchers. Current practices in hospitals or clinics are using steam with temperature above 100°C. This high temperature of steaming is obtained by applying a higher pressure and requires one hour for complete sterilization process [2].

Biocide technology can be divided into heat, steam, and chemical method [3]. These methods are mostly time consuming, high temperature requirement and, high pressure treatment [3]. It is also expensive, involves high electricity, and the application is only specific to certain material only. New approach plasma application in medical and industry bring a new expectation in sterilization technology. Plasma are proposed to be used to inactivation microorganism [1,4,5]. Plasma is an effective biocide device because of its short time treatment, harmless on human and wide application [1].

This paper aims to analyze the properties of atmospheric jet plasma especially to improve the technique and process of treatment. The common technique used to analyze plasma parameter is Lagmuir probe. Lagmuir probe used to determine the electron density, electron temperature, and electric potential of plasma [6]. Optical emission spectroscopy (OES) is also popular in study of temperature of excited electron and plasma density [7-9]. In this work, OES was applied in order to study of emission line spectrum in plasma. The reactive species in plasma flume which affect the inactivation process was captured directly using spectrometer.

Reactive species and atomic radicals are great interest in plasma chemistry. For example oxygen atom and hydroxyl radical are important in stimulated oxidation process [10]. Plasma chemistry can provide the information about mechanism and kinetic of chemical process in plasma [10]. This paper describes the probability chemical reaction happen on the reactive species in this plasma jet. In order to identify the species in emission, spectrometer was utilized in this experiment. These species can be identified by using wavelength in emission line spectrum. The emission spectrum was important to determine the critical plasma parameters in order to control a plasma process. Intensity in emission line is used to quantify the particle and electron densities [11]. From this experiment, plasma inactivation of microorganism can be analyzed.

II. BACKGROUND OF ATMOSPHERIC GAS PLASMA

The first atmospheric plasma was developed in the 1857 by W. Siemens for ozone generation using oxygen flow into dielectric barrier discharges (DBD) as a plasma source [12]. Since then, a large number of cold atmospheric plasma devices have been developed. In 1988, atmospheric pressure glows for discharge plasma was recognized as potential plasma system in medical field [4], food industry [13] and water treatment [14].

Cold atmospheric plasma has been shown suitable for object that are heat sensitive for instance, object in the liquid state, biological tissue or organic material [4,8]. Such system are suitable for disinfection and widely used to inactivate a microbial including spores [1], bacterial [15], cell cancer and

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tissue [8]. Other example includes the inactivation of *Escherichia coli* [16], *Candida albicans* [15], *Pseudomonas sp* [7], *Enterococcus faecalis* [17], and leukemia cancer cell [18].

Plasma jet can be generated by applying high amplitude voltage (HV) between two electrodes into gas source. Noble gases such as Helium (He), Argon (Ar), Xenon (Xe), or Neon (Ne) can be used as source of plasma gases. Plasma are formed under atmospheric pressure and temperature with gas of neutral atoms by removing an electron from atom, leaving a positive ion [12]. This process happens at high temperature and called as ionization process. Ionization process can be express as in (Eq. 1) reaction where, A is the atom, A^+ is the positive ion and e is the electron [19].

$$e + A \rightarrow A^+ + 2e \quad (1)$$

Plasma is categorized as a partially ionized gas containing neutral particles, free electrons (light), positive ions (heavy), atoms, energetic electrons, metastable particles, and UV irradiation [10]. Every single data on these species is significant in order to understand of the plasma physic and chemical reaction. Atmospheric plasma are produced by ionization and breakdown atom and molecule of the neutral species. The high energy from the outer shell of electron was removed by ionization energy by thermally, photon-irradiation, potential energy of excited species, or the kinetic energy of high speed electron process [14,19].

Normally researchers relate their finding using spectrum emission in order to analyze the mechanism of plasma treatment or the causes of the inactivation process [8,15,17,20]. For example, Q. Xiong et al. discuss the characteristics of plasma and electron density in helium plasma jet by relative and absolute OES [9]. T. Murakami et al. explains the probability of plasma chemistry in plasma helium mixed oxygen (He/O_2) with presence of humidity in open air experiment using species present in line emission [21]. Wei Chen et al. demonstrate He/O_2 plasma more effectively to kills bacteria compare to the pure helium plasma [17]. He indicates that OES analysis clearly shows that hydroxyl radical and oxygen radicals in plasma are mainly responsible for the bacteria death.

For this work, the reactive species present in cold atmospheric plasma jet was investigated using the emission line spectrum to recognize the probability the plasma chemistry occur in ionization and kinetic process. Two electrodes are connected to high voltage power and ground in order to produce spaces for ionization and collision of electron process.

The bacteria *Escherichia coli* was used as a model microbe and atmospheric plasma jet are presented as the key of mechanism of biocides devices. Then, the relative species role and responsibility are being discussed for inactivating agents.

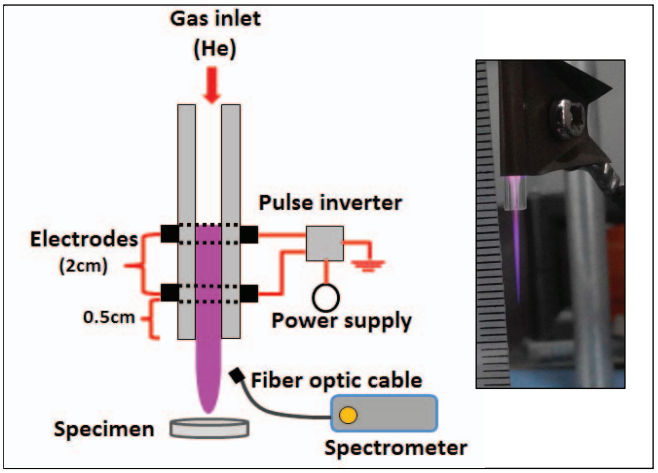

Fig. 1: Helium plasma jet setup experiment and plasma flume.

III. Experiment Arrangement and Procedures

A. Sample Preparation

Samples of *Escherichia coli (E.coli)* ATTC 25922, Gram Negative bacteria were obtained from Microbiology Laboratory, Tuanku Fauziah Hospital. Single colony of *E.coli* with concentration 0.5 McFarland were cultured at 37 ^oC for 18 hours in Mueller Hinton. Every colony was cultured at the same size and thickness in order to have homogeneous size for plasma treatment.

B. Helium Plasma Jet

The plasma device set up is shown in Figure 1 The set up composed of glass tube with inner diameter 1.5 mm, pulse inverter, glass tube and two electrodes. The plasma device was connected to helium gas channel through the Teflon tube and a glass tube. Voltage 16.6V were supplied to pulse inverter. The two electrodes were placed in a glass tube and connected to a pulse inverter which used to convert voltage supply into high voltage. The gap between the two electrodes is 2 cm and the position between glass tip and the specimen was 0.5 cm. Helium gas was controlled using mass-flow controller system for accurate flow rate and timing exposure to sample. This gas system included the flow meter system, valve, power system, control system, gases and Labview® software.

Plasma jet is able to generate plasma flume proportional to the value of the flow rate. The diameter of plasma glow is less than 1.5 mm. Although the plasma jet was relatively small size, it was appropriate for our purpose research in understanding the microbial inactivation.

C. Optical Emission Spectroscopy

To analyze the plasma spectroscopy, OES was used. The EPP2000-HR High Resolution spectrometer from Stellar with sensitivity for a wavelength between 190 nm to 1100 nm was used to collect emission data. To collect the light, fiber optic probe (UV-VIS-NlR) with 600 µm was held at 45 ° and 0.5 cm away from plasma glow. The spectrum was observed using SpectraWiz OS v5.3(c) 2013 software.

IV. Result and Discussion

A. Emission Line for Reactive Species in Plasma Jet

The typical OES spectra of the plasma jet were obtained using 99.9% pure helium gas at atmospheric pressure. The emission spectrum was recorded within the range 200 nm to 900 nm. The flow rate was varied 400 ml/min, 600 ml/min, 800 ml/min and 1000 ml/min with voltage supply 16.5 to 16.6 V. Figure 2 to Figure 5 shows the optical emission spectrum of plasma jet between 200 nm to 900 nm. This comparison was conducted to investigate reactive species in different flow rate during *E.coli* inactivation based on OES measurement.

Table I shows optical emission lines measured from plasma flume. The emission spectra are dominated by helium (He), nitrogen (N_2), and oxygen (O_2) species. The compositions of species in each flow rate were present at the same molecular and atomic molecule emission lines but at different relative intensities. A strong emission appears in range 300 nm to 400 nm with nitrogen species. Helium appears above the 400 nm line both at lower intensity. For line emission below than 280 nm, very little emission appears, confirming that the UV photons were minor in atmospheric plasma glow. Helium line emissions emitted between wavelengths 356.81 nm to 666.88 nm. A possible assumption for the excitation process of helium is as in (Eq. 2) [22].

$$He + electron \rightarrow He^*(2^3S, 2^1S) + electron \quad (2)$$

Although the experiment was carried in open air, He* is not significantly affected by the surroundings. He* is influenced by Penning ionization to produce He molecule [21].

$$He^* + O \rightarrow He + O^+ + e \quad (3)$$
$$He^* + O^2 \rightarrow He + O^* + O \quad (4)$$

Due to open air experiment, humidity caused additional species such as oxygen (O) atom and hydrogen (H) atom to be observed. Nitrogen (N_2) molecule and oxygen (O_2) molecule are produced from the present of atmosphere [21]. Nitrogen molecule, N_2 ($C^3\Pi_u \rightarrow B^3\Pi_g$) [8] appear in wavelength line emission from 300 nm to 400 nm. In this experiment, N_2 appear at almost the same wavelength in every flow rate, starting form 308.96 nm until 776.19 nm. The N_2 species production processes in open air are the electron induces processes [21].

$$e + N_2 \rightarrow N + N + e \quad (5)$$

N_2^+ ($B^2\Sigma_u^+ \rightarrow X^2\Sigma_g^+$) is positive ion where it can occur in helium plasma with ground state of nitrogen in open air experiment [8].

$$e + N_2(X^1\Sigma_g^+) \rightarrow e + N_2(C^3\Pi_u) \quad (6)$$
$$e + N_2(X^1\Sigma_g^+) \rightarrow e + N_2^+(B^3\Sigma_u^+) \quad (7)$$
$$He^* + N_2 \rightarrow N_2^+ + He + e \quad (8)$$

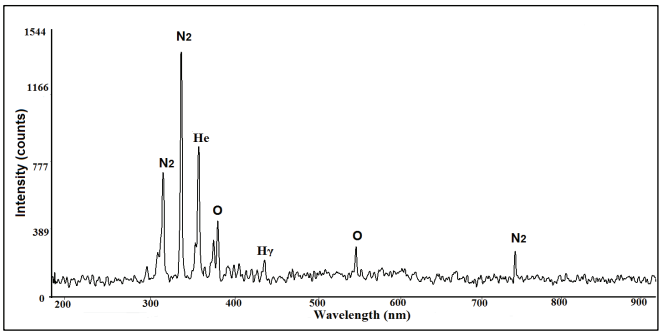

Fig. 2: Emission spectrum of plasma He flow rate, 400 ml/min; range 200 nm to 900 nm

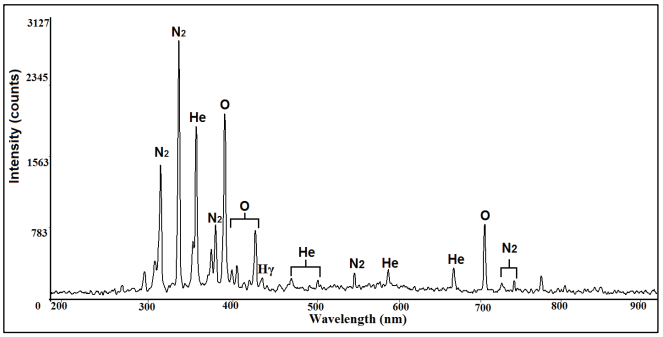

Fig. 3: Emission spectrum of plasma He flow rate, 600 ml/min; range 200 nm to 900 nm.

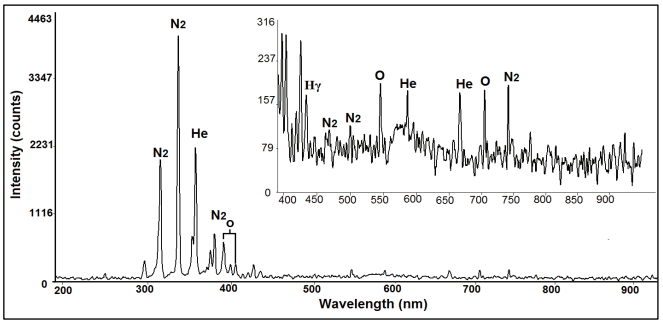

Fig. 4: Emission spectrum of plasma He flow rate, 800 ml/min; range 200 nm to 900 nm

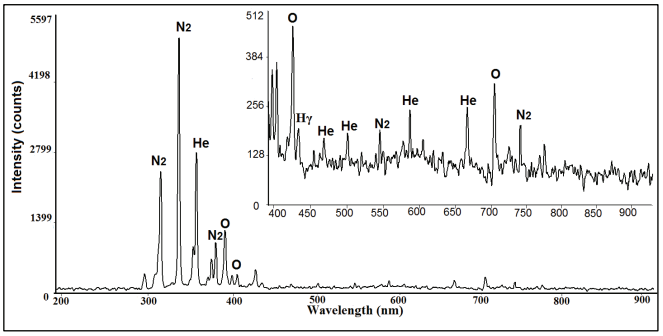

Fig. 5: Emission spectrum of plasma He flows rate, 1000 ml/min; range 200 nm to 900 nm.

TABLE I. LINE EMISSION SPECIES IN PLASMA FLUME [23].

Flow rate ml/min	Species	Emission lines (nm)
400	He	356.81 ($2^3P \rightarrow 10^3S$)
	N_2	308.52 (N III), 315.08 (N V), 336.57 (N III), 546.02 (N II), 742.07 (N I)
	O	379.76 (O II), 405.03 (O II)
	Hγ	434.86
600	He	356.81 ($2^3P \rightarrow 10^3S$), 469.76 ($3^2P \rightarrow 4^2S$), 501.69 ($2^1S \rightarrow 3^1P$), 586.99 ($2^3P \rightarrow 3^1D$), 666.88 ($2^1P \rightarrow 3^1D$)
	N_2	308.52 (N III), 315.08 (N V), 336.57 (N III), 379.76 (N III), 545.57 (N II), 726.69 (N II), 741.60 (N III)
	O	390.39 (O III), 426.83 (O II), 705.31 (O IV)
	Hγ	434.86
800	He	356.81 ($2^3P \rightarrow 10^3S$), 468.86 ($3^2D \rightarrow 4^2P$), 586.99 ($2^3P \rightarrow 3^1D$), 665.96 ($2^1P \rightarrow 3^1P$)
	N_2	308.96 (N III), 315.08 (N V), 336.13 (N III), 379.32 (N III), 463.48 (N III), 500.34 (N II), 742.07 (N I), 776.19 (N II)
	O	374.46 (O IV), 390.39 (O III), 546.02 (O II), 705.31 (O IV)
	Hγ	434.86
1000	He	356.81 ($2^3P \rightarrow 10^3S$), 468.86 ($3^2D \rightarrow 4^2P$), 501.24 ($2^1S \rightarrow 3^1P$), 586.99 ($2^3P \rightarrow 3^1D$), 666.88 ($2^1P \rightarrow 3^1P$)
	N_2	308.52 (N III), 315.08 (N V), 336.13 (N III), 379.76 (N III), 545.57 (N II), 742.53 (N I)
	O	398.81 (O II), 405.03 (O II), 426.83 (O II), 705.31 (O IV)
	Hγ	434.41

This plasma experiment gives largest contribution in production of oxygen species. Oxygen species in this plasma emitted at line spectrum 400 nm to 742 nm. Since the helium species and charge particle take place in ambient pressure, they can pass their kinetic energy to the ground state oxygen molecule [24]. These processes produce the reactive oxygen species with the presence of oxygen molecule in glass tube. Oxygen species may be driven by two induces process which generates oxygen molecule (O_2) or ozone (O_3) by electron induced process as in (Eq.9) or heavy particle induced process as in (Eq. 10) [21].

$$e + (O_2 \text{ or } O_3) \rightarrow O + (O \text{ or } O_2) + e \quad (9)$$

$$O_2 (^1D) + O_3 \rightarrow O + O_2 + O2 \quad (10)$$

Humidity in open air can generate the hydrogen, Hγ ($^2P^0 \rightarrow ^2D$) molecule which is presence at basic line at 434 nm. The dissociation of water (H_2O) are excited by electron to produce the Hγ atom [21, 25]. Hγ also can react with oxygen molecule to generate OH.

$$e + H_2O \rightarrow OH + H + e \quad (11)$$

$$H + O_2 \rightarrow OH + O \quad (12)$$

$$H + O + He \rightarrow OH + He \quad (13)$$

Continuous collision process makes further collision of reactive species of OH and produce hydrogen molecule [30].

$$OH + O \rightarrow H + O_2 \quad (14)$$

There are possibly have a few week NO species molecular γ-bands in line 200 nm to 300 nm due to chemical reaction of N_2 and O_2 [25]. This reaction can cause by humidity in air.

$$OH + N \rightarrow NO + H \quad (15)$$

$$O + NO_2 \rightarrow NO + O_2 \quad (16)$$

$$H + NO_2 \rightarrow NO + OH \quad (17)$$

B. Inactivation of Bacteria E.coli ATTC 25922

Bacteria *E.coli* ATTC 25922 was exposed to plasma for 5, 10 and 15 mins. The bacteria *E.coli* were treated for every single colony. Each treatment was repeated three times. After being exposed to plasma, *E.coli* was re-cultured in Mcconkey medium agar to analyze the efficiency of treatment. The results were observed 24 hours after exposure. Table II shows result of *E. coli* growth after being exposed to plasma. There are grown of *E.coli* at flow rate 400 ml/min with time exposed 5 mins and 10 mins, but no growth of *E.coli* at 15 mins. For flow rate 600, 800 and 1000 ml/min, it also showed no growth.

There are variation for each flow rate helium gas and OES measurement. Regarding to the emission line spectrum of plasma helium, the volume of reactive species appears in flow rate 400 ml/min are lower compared to other flow rate. Low of reactive species in plasma requires longer disinfection time. This can be discovered where effectiveness of treatment only take place at 15 mins for flow rate 400 ml/min. However, at other flow rates, complete inactivation was accomplished in 5 mins. In line emission spectrum, intensity of every species also different for each flow rate. For example, nitrogen at higher flow rate has higher intensity compare to other flow rate. Helium flow rate 400 ml/min has the lowest intensity for every species presence in plasma flume. It can be determine that intensity contributes in inactivation performance, where longer times are required to inactivate the bacteria *E.coli* if intensity of species is low.

TABLE II. INACTIVATION OF E.COLI ATTC 25922 AT DIFFERENT TIME AND FLOW RATE

Flow rate ml/min	Exposure time for each colony		
	5 minutes	*10 minutes*	*15 minutes*
400	Growth	Growth	No Growth
600	No Growth	No Growth	No Growth
800	No Growth	No Growth	No Growth
1000	No Growth	No Growth	No Growth

C. Reactive Species for Inactivation of Bacteria

Plasma sterilization is a complex process since many plasma reactive species are present. Reactive species are generated through various collisional processes, such as electron impact excitation, rotational energy transfer (RET), and vibrational energy transfer (VET) [21, 20]. Researchers state that reactive species are very important in plasma sterilization [8, 16].

In this experiment, *E.coli* sample produces stink smell when expose to plasma. This smell are identified as volatile organic compound (VOC) which caused by chemical reaction of reactive species that embedded into cell wall [26]. VOC were produced by the breaking chemical bond on microbe atom by atom through etching process and erosion of atom by atom of microorganism [16]. Nitrogen species were frequently observed in emission spectrum with high intensity compare to oxygen species in line 300 nm to 400 nm. Due to strong oxidation process, nitrogen can relate to reactive nitrogen species (RNS) effect. RNS effect can be described with the formation of charge species such as NO, N_2^+, and NO_2 [9, 21].

In the inactivation process, nitrogen reactive species are embedded into membrane cell and further chemical reactions will take place inside them. This chemical reaction causes the cell to unable to generate a food. This causes cell death. Starting from emission line 400 nm until 800 nm, oxygen molecule plays a role in inactivation of microbes. Oxygen has low intensity compare to nitrogen. Basically, oxygen species are related to the reactive oxygen species (ROS) effect. ROS species are diffused into bacteria biofilm and kill them from inside cell by breaking the chain of amino acid inside protein [27].

RNS and ROS acts together to inactivate the microbe. Strong oxidizing process of reactive species can make component in bacteria to replicate abnormally such as DNA mutation [28]. DNA contains of protein and if mutations happen on DNA, it can lead to protein defects [28]. In theory, the damage of DNA, RNA and protein also can contribute to physical ageing of microbe [29].

E.coli which is gram negative are easier to get deactivated by plasma compare to gram positive microbe [1,8]. *E.coli* is prokaryotic microorganism, so the wall structures are much thinner and simple compare to eukaryote microbe. *E.coli* cell wall contains protein, phospholipids and lipopolysaccharide [30]. These lipid bilayers are unsaturated fatty acids which act as barrier to prevent ion or compound in and out of the cell [20]. For reactive species such as hydroxyl radicals, it is able to break unsaturated fatty acids at cell wall [20]. The reactive species explain above give direct impact and chemically attack on the microbe especially on outermost membranes. The production of NO and NO_2 in plasma also adds to the lethality of the inactivation bacteria.

Charged particles such as electron and ions also important in the rupture and disruption of outer membranes [26]. Charged particles induce an electrostatic tension and cause rupture of chemical bond which affect to tensile strength of membrane [28]. The membranes also become erosion and lesions when reactive species together diffusion into cell. This process are only effective on gram negative bacteria, because it has thin cell membrane [31]. Gram positive species was lack an outer membrane but have a denser murein layer (peptidoglycan layer) thereby these bacteria has advanced strength and inflexibility membrane cell [31]. There are also several investigation proposed UV radiation was one of the inactivation mechanisms [4]. UV radiation can be found in line emission from 200 nm to 300 nm. UV functions to inactivate the genetic material such as DNA by quick absorption. In cold plasma, UV radiation not significantly important due to power density of UV are low [20].

In future analysis, additional device such as microscopic observation can be applied to observation the structural damage suffered by the bacteria when exposed to the plasma. Scanning Electron Microscopy (SEM) or Transmission electron microscope (TEM) is famous device by researches to analyze the effectiveness of plasma treatment on microbe structure [8]. The density of electron such as helium metastable state or other species can be measure using advance laser absorption spectroscopy or using Stark broadening [9]. Fluorescence thermometer can be employed to measure the temperature of neutral gas [22]. Additional study for UV photon also can be analyze using UV fused silicon lens where it allowed light transmission to hit until lower than 185 nm and blocked other charge and neutral species [24].

V. Conclusion

In summary, this experiment demonstrated the effective inactivation process using atmospheric plasma jet. The inactivation was influenced by flow rate of helium gas. The higher flow rate, the more effective the inactivation performance becomes at shorter treatment time. The amounts of the reactive species also depend on the flow rate of the basis gas. There are presences of oxygen and hydrogen atoms which cause by humidity in air. Nitrogen and oxygen molecule are generated due to atmosphere condition. It is found that the nitrogen has high intensity compare to the oxygen species. The inactivation of bacteria takes place around emission line 300 nm to 450 nm with nitrogen as major reactive species. RNS and ROS in plasma are the main agents by damaging the inner cell of microbes.

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