Study on Bovine Bone Surface After Atmospheric Plasma Treatment

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Abstract

This paper studies of the effect on surface characteristics of bone surface after plasma treatment. For that purpose, the experimental procedure is designed to develop atmospheric plasma jet device. The physical processes such as electron dynamics, electron field, ionization and dissociation in relation to atmospheric pressure plasma are stated. The plasma jet is ignited in a glass tube with helium gas flowing freely into the surrounding air. Some consideration to develop the plasma jet is made to get stable plasma. In addition, its focuses on the surface analysis for bone surface after being treated to plasma. A few samples of freeze dried bovine bone were radiated in plasma. Then, the surfaces of bone were characterized in the term of surface morphological changes by using Scanning Electron Microscopy (SEM) and the data were analyzed to observe it differences. From the results of SEM, we could confirm that the main phenomena such as the reactive etching has modified the surface morphology.

Keywords

Surface characteristics, bovine bone, atmospheric plasma treatment and surface analysis.

I. INTRODUCTION

Plasma can be defined as an ionized gas with a mixture of electrons, negatively and positively charged particles of equal number, neutral atoms and molecules. It can be referred as the fourth state of matter, apart from the solid, liquid, and gas states where molecules become more energetic and transform matter in the sequence as the temperature increases[1, 2].

The plasma can be divided to two types which is the high temperature and low temperature plasma. In the other words, high temperature plasma also known as thermal plasma which in a thermal equilibrium condition or also called 'local thermal equilibrium' (LTE), all species such as electrons, ions and neutral atoms, their temperature is exactly same while the low temperature plasma or cold plasma is not in thermal equilibrium, called as 'non-local thermal equilibrium' (non-LTE) implies that the temperature of all plasma species are different with each other [3].

Table 1.1 shows the main differences between LTE and non-LTE plasma [3].

Table 1: The main differences of LTE and non-LTE plasma [3]

Type	LTE plasmas	Non-LTE plasmas
Current name	Thermal plasmas	Cold plasmas
Properties	$T_e = T_{h\backslash}$ High electron density: 10^{21}-10^{26} m^{-3} Inelastic collisions between electrons and heavy particles create the plasma reactive species whereas elastic collisions heat the heavy particles (the electrons energy is thus consumed)	$T_e >> T_h$ Lower electron density: $< 10^{19}$ m^{-3} Inelastic collisions between electrons and heavy particles induce the plasma chemistry. Heavy particles are slightly heated by a few elastic collisions (that is why the electrons energy remains very high)
Example	Arc plasma (core) T_e=T_h ~ 10 000 K	Glow discharges Te ~ 10 000- 100 000 K Th ~300- 1000 K

At 18th century, a mathematics professor at Gottingen University in Germany, G. C. Lichtenberg (1752-1799) has discovered beautiful brush-like patterns on insulating surfaces following discharges from a pointed electrode which become the earliest roots of plasma science. However, the first attempt to explain such phenomena came from Michael Faraday (1791-1867), the discoverer of electromagnetism which well-known exploding-wire experiments were clear precursors of the 'fourth state'. At the same time, Sir William Crookes (1832-1919) observed the glow when he applied voltage across electrodes in a partially evacuated glass tube [4].

In the late 1850s, the 'biological' application of gas plasma was first introduced by a German engineer, Werner von Siemens when he used a dielectric-barrier discharge to generate ozone from molecular oxygen in order to clean water from biological contaminants, even though he was not realized the underlying science of interaction between plasmas and biological cells at that time and there is no research made to understand about it. After 130 years later, some efforts were made to use plasmas for biological sterilization until the atmospheric non-equilibrium plasmas become a hot research topic, which investigations aimed at plasma-related phenomena and its use in modern technologies [4, 5].

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II. LITERATURE REVIEW

To enhance surface characteristics, surface treatments had been done before such as flame and corona but recently, plasma can be applied to improve wettability and adhesion [6, 7]. In the past, the majority of plasma processing has been done at low pressure in a vacuum chamber and viewed as a necessary processing requirement but recently, studies on the surface treatment of various material are concentrated on the atmospheric pressure by using helium or oxygen plasma instead of low pressure plasma due to the various advantages of atmospheric pressure plasma [7, 8].

A. Plasma Generation

Plasma can be generated by applying energy to a neutral gas causing the formation of gas carrier. Then, electrons or photons with sufficient energy will collide with the neutral atoms and molecules in the feed gas (photoionization) to reorganized it, thus produced excited species and ions in the gas phase. This collisions of gas involves elastic and inelastic collisions. However, the elastic collision only rise their kinetic energy while the inelastic collision is important to produce the excitation and ionization process when the collision are energetic enough [3, 9, 10]. Plasma ignition used electric energy due to its availability as well as maintaining the plasma state. So, electric field can be produced either by an alternating current or by a direct current supply [1].

B. Plasma Medicine

Prof. Dr. Klaus-Dieter Weltmann, Director of The Leibniz Institute for Plasma Science and Technology said that plasma medicine can be divided into three research fields based on plasma decontamination and cell treatment which is surface modification, biological decontamination and therapeutic application [11].

Surface modification can be defined as the act of modifying the surface characteristics to create a new surface with particular surface properties such as adhesion, biocompatibility, roughness, hydrophilicity, surface charge, surface energy, absorptivity and reactivity over the original surface. In addition, surface modification of a material can improve its biocompatibility without changing its bulk properties.

Plasma used in biomedical application for a few reason: (1) reactive plasma processing allows treatment of different materials (e.g. cleaning, activation, etching, coating), (2) plasmas exhibit excellent gap penetration, (3) plasma processes allow very low thermal substrate load, (4) plasma processes allow specific surface modification [12].

C. Plasma Source.

Plasmas can be obtained by using a different sources but the familiar ones are the gaseous, metallic and laser-based plasma sources.

Gaseous Plasma Source. A gaseous plasma is created by supplying a potential through the gas where the breakdown potential is influence by the pressure and discharge gap width. There are many kinds of low-pressure plasma sources, such as direct current (dc), radio frequency (RF), and electron cyclotron resonance (ECR) plasma sources. These type of gaseous plasma operated at low-pressure since the breakdown electric field is smaller and the current is more controllable. Also, a large area uniform plasma with a well controlled electron density can be produced by these plasma sources. Beside that, a corona discharge and arc plasma at atmospheric pressure are also used in biomedical engineering [13].

Vacuum Arc Plasma Source. A vacuum plasma arc consists of cathode in the form of a plate with a large surface area. This plasma source (the arc evaporators) can be used in technologies for ion-plasma deposition of coatings but it have an important disadvantage that there is a high quantity of droplets exists in the cathode erosion material. However the new invention could overcome this problem since pulsed vacuum-arc plasma source is operating in the reflective discharge mode [14].

Laser Plasma Source. Formation of the plasma is generated by the process of laser plasma interaction when a solid target is exposed to the laser, absorbed it, thus produces its breakdown where the matter evaporated and heated into plasma. At the same time, the temperature of plasma can easily be in excess of 10^8 K while the high laser fluence focussed intensities onto target in excess of 10^{20} Wcm^{-2}. A sample that can absorb laser radiation such as metallic and composites target can be used with gas feeding to form gaseous plasma. In the process of the laser-induced plasma, there are two main mechanisms involved. The first one is multiphoton ionization and then electron impact which leading to vapor breakdown and material ablation [13, 15].

Atmospheric Pressure Plasma. Nowadays, plasma surface treatment at atmospheric pressure has been developed aggressively. It has been applied to several polymeric materials for adhesion improvement [16]. At atmospheric pressure, plasma are weakly ionized and the mean-free path for electron-molecule collision is very short, <1μm [17]. Their advantages over vacuum system are they do not require expensive and complicated vacuum equipment thereby reduced the cost and also easy to build and operate [7, 17, 18].

Atmospheric pressure plasma also can be classified into two distinct categories which are thermal plasma and cold plasma. Plasma torches and those produce in high intensity arcs and high frequency discharges are thermal equilibrium plasmas. For low temperature plasma, the temperature in the plasma reactor stays near room temperature and because of that reason, these plasma are said cold plasma. As mentioned before, the electron temperature T_e for nonthermal plasma is very high but the sensible temperature, T_h remain ambient. It's very reactive although have a low degree of ionization and low density of charged species despite have a high density of activated species such as excited state atoms and free radicals [19]. The application in the wide variety of biomedical aspect of atmospheric pressure plasma is presented in the following section.

There are a few techniques used to energize the gas of atmospheric non-thermal plasmas such as corona discharges, atmospheric pressure plasma jet (APPJ), dielectric barrier discharge (DBD) and micro hollow cathode discharges (MHCD) [20]. However, one of the problem that have been found while generating glow discharge are instabilities, particularly glow to arc-transitions causing the filamentation of the glow discharge in short times than the desired lifetime of a homogeneous flow [21]. According to that, by comparing the source of gaseous plasma, a plasma jet is the most useful techniques designed to prevent arching which normally created in a quartz capillary tube constructed with the double dielectric electrode configuration in order to produce dielectric barrier discharge (DBD) [22, 23].

III. METHODOLOGY

The flow diagram of the project is illustrated in Figure 1 below.

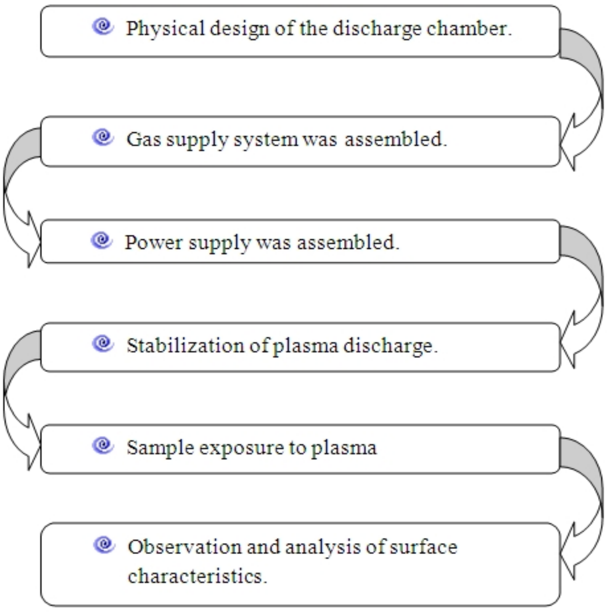


Figure 1: The flowchart for the whole project

Firstly, physical design of the discharge chamber is considered where a cylindrical hallow casing of quartz glass with two cuprum electrodes were designed. Then, gas supply systems were assembled from helium gas, pressure regulator, pressure gauge, valve, outlet connection, mass flow controller, and gas tubes. Helium gas is used due to it principle effect to excite plasma where it posses low breakdown voltage. At the same time, power supply will be assembled from ACDC converter, and current regulator. The gas flow, electrodes distance, and voltage were adjusted to stabilize plasma discharge.

In this project, the experiment of generating the atmospheric pressure plasma jet is prepared and arranged which is similar to Figure 2 where it shows the design of the device schematically. The environment of experiment is in atmospheric pressure (1 atm or 101.3 kPa) and at room temperature (27° C or 300 K).

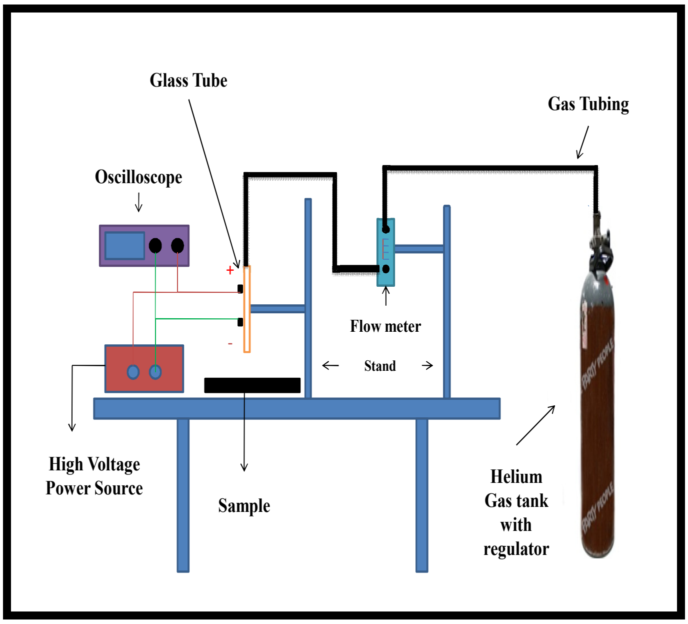


Figure 2: Schematic Diagram of Experimental Setup

The distance between two copper electrodes is 25 mm whereas the gap from the mouth jet to the bone surface is about 5 mm as shown in Figure 3 below. Finally, three samples of bovine bone were exposed to plasma for about 5 minutes and evaluation of the effect to the surface of bone was carried out. The bone is tested on the improvement of surface morphology by means of surface morphology of the bone surface by using Scanning Electron Microscopy (SEM).

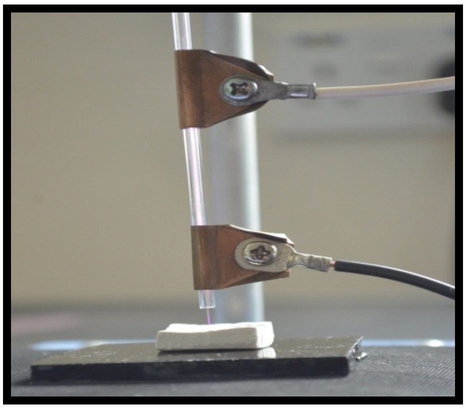


Figure 3: Establish the position of the sample to 5 mm from the mouth jet and the distance between electrodes is 25 mm

IV. RESULT

A. Design of Atmospheric Pressure Plasma Jet Device

Figure 4: The Atmospheric Pressure Plasma Jet Device

An atmospheric pressure plasma jet device is developed as shown in Figure 4 above. Shown in Figure 5 is the glow discharge plasma generated from the jet mouth. The dielectric electrodes made of copper foil wrapping the glass tube and the gap between the electrodes is about 30 mm. The ground electrode is on the upstream side; the active electrode is on the downstream side and 10 mm apart from the tube orifice.

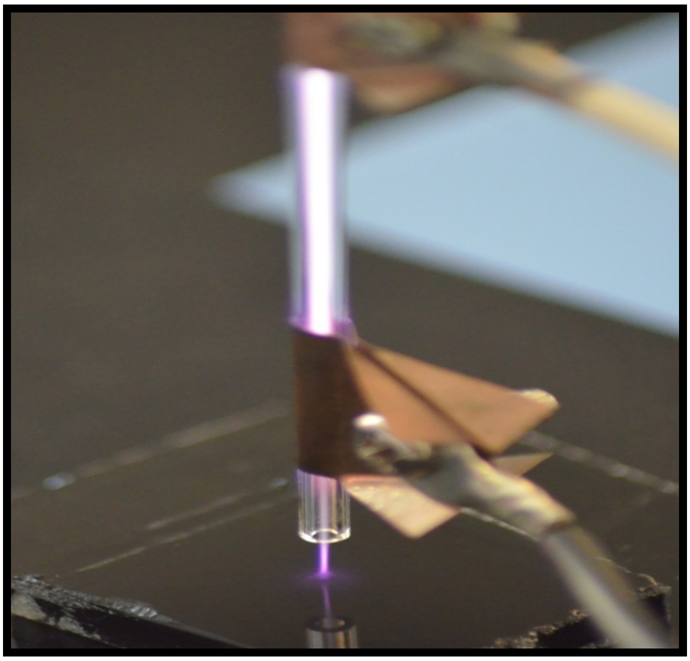
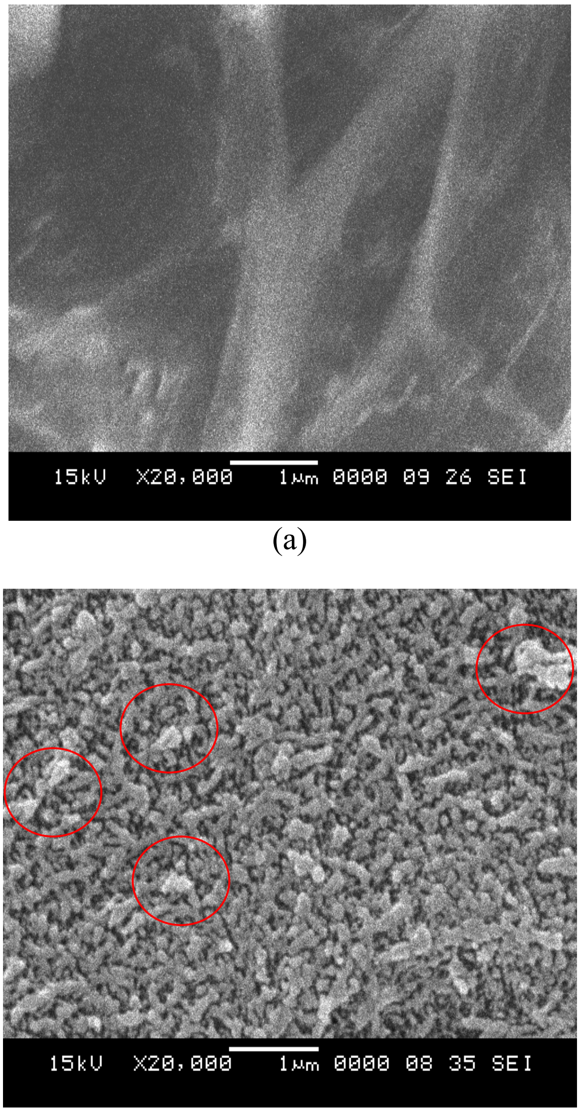


Figure 5: The Glow Discharge Plasma Generated

B. SEM Images and Analysis for Bovine Bone

The JSM 6460LA Scanning Electron Microscopy (SEM) was used to inspect morphological change of the bovine bone surface after plasma treatment. Scanning is conducted at the center where the plasma is charged and the side surface of the bone to know whether the plasma react with other parts or not.

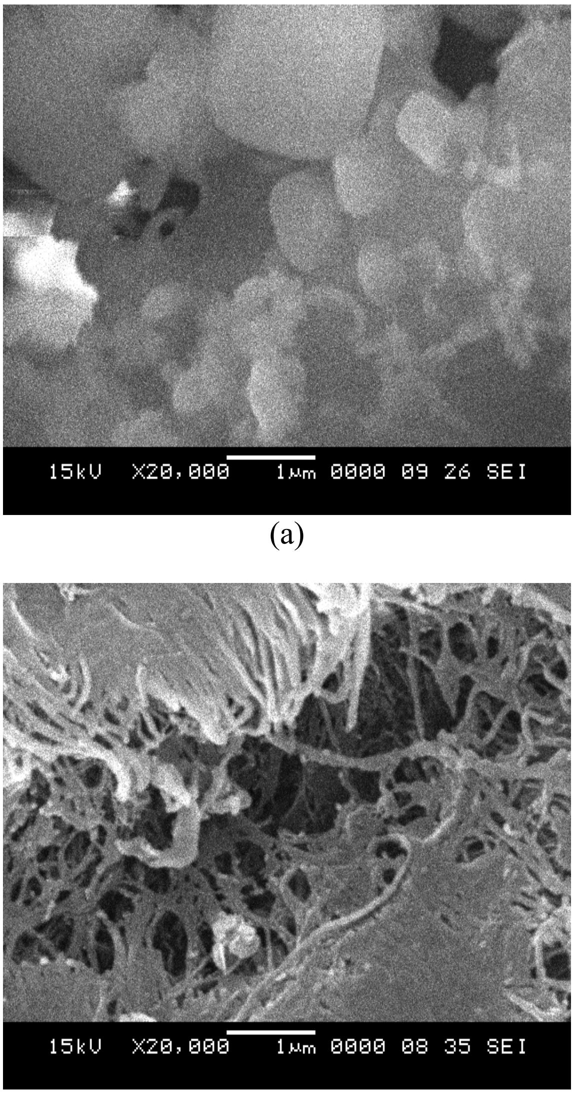


(a)

(b)

Figure 6: SEM micrograph of cortical bovine bone (a) control sample; (b) plasma treatment at the inside surface of the bone sample

From SEM images focus at center of the bone surface shows in Figure 6 above, comparing treated and untreated sample at a treatment time of 5 minutes for a largest magnification, the obvious change between the samples is that the surface area increased where for sample (b), we can see the effect of etching with a depth of 1µm at the highlighted areas.



(a)

(b)

Figure 7: SEM micrograph of cortical bovine bone (a) control sample ; (b) plasma treatment at the inside surface of the bone sample

From SEM image focused at the side of the bone surface shown in Figure 7 above, comparing treated and untreated sample at a treatment time of 5 minutes, the obvious change between the samples is that the surface area increased where for sample (b), we can see the morphology of the surface drastically changed. Although the plasma flame is subjected to the center of surface, we still can see the effect of plasma at the side of the bone surface.

Overall results showed that the surface area of treated samples dramatically increased as well as the etching effect and the surface morphology was enhanced. It is proven that plasma treatment gives an effect to the surface characteristics of bovine bone. Therefore, adhesion property of bone surface can be improved.

V. DISCUSSION

The plasma were characterized based on type of gas, gas flow, distance between electrodes, the voltage applied, and temperature of the plasma. This kind of factors basically will influence the length of the plasma generated, thus stable plasma can be achieved. This is proven through the adjustment of electrodes distance. To prevent arching, the gap of electrode must be 10 mm above.

At atmospheric non-thermal plasmas, the produced plasma are glow discharges where gas is heated very little above room temperature (1/40 eV) and electron energies as high as 10's of eV. According to Paschen curves, breakdown voltage increase with the product of gas pressure and electrode separation. Besides, breakdown voltage is very high and its mechanism is often streamer breakdown (spatially non-uniform) at atmospheric pressure. Furthermore, glow to-arc-transition can develops from a small perturbation and it is difficult to stabilize a glow discharge because the transition time is very short. Therefore, to avoid the risk of arching, an inert gas (helium gas) is used to lower the breakdown voltage. High frequency power source also can prevent an arc other than the use of dielectric barrier discharge mode and structured cathode.

The reasons to use a noble gas especially helium are as follows: (1) low ignition voltages (e.g. helium: 4 kV/cm); difficulty in developing to an arc discharge, (2) high gas thermal conductivity (as high as that of H_2 gas), prompt removal of the generated Joule heat, (3) long lifetime of meta-stable excited atoms (helium 2^3S, 2^1S), and highest electron energy level among all materials, easy dissociation of mixed gas molecules, and generation of ions and radicals, and (4) no formation of compounds with mixed monomers, as a noble gas is inert [24].

Table 2: The ionization potential (energy) of noble gases [25].

Gas	Ionization Potential (eV)	Gas	Ionization Potential (eV)	Gas	Ionization Potential (eV)
He	24.5874	H_2	15.4259	CO_2	13.723
Ne	21.5645	D_2	15.46	CH_4	12.71
Ar	15.7596	O_2	12.07	NO	9.264
Kr	13.9996	N_2	15.581	N_2O	12.886
Xe	12.1299	CO	14.014	NH_3	10.2

According to the Table 2 above, the ionization energy of helium gas is 24.5 eV, which is the highest among all gases includes Neon, Argon, Hydrogen and Nitrogen. Helium gas also shows the lowest ignition voltage of 4 kV/cm compared to other gases. This happens because there is a long-lifetime metastable state of helium at the energy level of 20 eV which is just below the ionization energy. Furthermore, ionization actually needs approximately 4 eV, the differences of these levels in the continuous operation of the discharge. Besides, the helium discharge can become glow even in DBD because the applied electric field in helium plasma is extremely low, thereby the transition to the streamer requires a longer time than usual, which finally enhanced the diffusion of particles parallel to the electrode surface [24].

VI. CONCLUSION

As a conclusion, an atmospheric pressure plasma jet device that needs to be designed is studied and also characterized to evaluate the device capability for bone surface processing. The glow discharge of plasma in helium gas was driven by pulse excitation and high voltage must be supplied to the electrodes to make it happens. For surface analysis, Scanning Electron Microscopy is used to observe the surface morphological changes and through comparison with a control, we can see that the surface morphology was enhanced by means of surface area increased. Thus, plasma processing has proven to dramatically change surface morphology of bone surface. Moreover, the use of atmospheric pressure plasma in surface modification give more benefits in the form of biomedical aspect includes low environmental impact, no line-of-sight problem as compared to laser and UV radiation.

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