CHAPTER 1

Introduction

1.1 Introduction

Plasma is an ionized gas which can be generated under high pressure, atmospheric and low-pressure conditions. Atmospheric-pressure plasma is commonly generate in the kHz regime by corona discharge (CD) or dielectric barrier charge (DBD) in the radiofrequency (RF) regime by inductive coupled plasma (ICP) or non-thermal plasma jet (NTPJ), or technically cold atmospheric pressure plasma jet (CAPPJ). An NTPJ source consists of a center electrode and a grounded outer electrode [1, 2]. Pressured gas flowing between the electrodes is ionized and ejected from the source exit. The generated plasma contains chemical species, charged species, free radicals, heat and ultraviolet (UV) emitting species in different concentrations. The concentrations of the reagents are depending on the process parameters and the gas used [3].

The number of potential applications of non-equilibrium atmospheric pressure discharges in biology and medicine has grown significantly in recent years [4]. In fact, the activity in this direction has led to the formation of a new field in plasma chemistry titled ‘Plasma Medicine’. Some examples of medical applications of plasma are the use of plasma in the treatment of dental cavities [5,6], sterilization of various surfaces [7,8], treatment of skin diseases [4,7,8,9], delicate surgeries [7,10,11] and many other applications. Non-equilibrium atmospheric pressure plasma jets (APPJs) are of intense interest in current low- temperature plasma research because of their immense potential for material processing and biomedical applications.

The rapid development in the applications in biomedicine has generated the need for fundamental studies on mechanisms of plasma interaction with living tissues, cells and DNA. Moreover, clinical applications such as skin treatment using CAPPJ have made it necessary to determine possible deleterious effects on human tissues. The mechanisms of the damage are quite complex. Besides studying the CAPPJ effect on cells, researchers have also investigated its effect directly on DNA [12]. Damage to cellular DNA induced by ionizing radiation can occur by direct ionization of the DNA or through reactions with species liberated by primary ionizing radiation including solvated electrons, hydroxyl radicals and other radicals [13]. Such damage can result in biochemical changes within the DNA, including single strand breaks (SSB) and double-strand breaks (DSB). The latter is considered to be more problematic since it is harder to repair and thus often leads to cell death or mutagenesis. Previous research [12] found that DNA damage was particularly related to the oxygen concentration in the plasma. However, in CAPPJ a number of physical agents exist, such as ions, electrons, gas species, charge, neutrals, radicals, UV and even heat, and all of these agents may play their own certain role in damaging DNA. In this study, we tried to investigate separate effect of some of these agents on DNA change. Since the oxygen effect has been investigated [12] and because the helium plasma jets are routinely used in clinics, this research examined the effect of helium gas alone.

1.2 Literature Review

1.2.1 Cold Atmospheric Pressure Plasma Jet

Plasma has recently been applied to medical and dental applications such as using plasma for sterilization. People use plasma jets to treat infected wound, after that use a polymer film which is treated by plasma deposited onto the sterilized wound. In dental application, dental materials are treated by plasma. People use plasma to increase efficiency of denture so that denture can be used for longer. The number of potential applications of non-equilibrium atmospheric pressure discharges in biology and medicine has grown significantly in recent years. It is now clear that these plasmas can have not only physical but also medically relevant therapeutic effects—plasmas can trigger a complex sequence of biological responses in tissues and cells.

The energy of ions in plasma is generally significantly low compared with that of accelerated ion beam. Study of low-energy ion bombardment effect on biological living materials is of significance. High-energy ion beam irradiation of biological materials such as organs and cells has no doubt biological effects. However, ion energy deposition in the ion-bombarded materials dominantly occurs in the low-energy range and some applications involving ion beam and plasma use low energy ions.

1.2.2. Plasma interaction on DNA

Low-energy ion beam bombardment has been used to mutate a wide variety of plant species. Researchers have explored the indirect effects of low-energy ion beam on biological damage due to the free radical production in plant cells [7]. Free radical production is regarded as the main source of damage to cells under biotic and abiotic stresses [8]. Some species of free radicals are highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins, nucleic acid, etc. Damage to cellular DNA induced by ionizing radiation can occur by direct ionization of the DNA or through reactions with species liberated by primary ionizing radiation including solvated electrons, hydroxyl radical and other radicals [5].

Previous study of *Thopan*, et al published that the ion bombardment with energy as low as several-tens eV was possible to break DNA strands and thus potential to cause genetic modification of biological cells [9].

In the same way, in cold atmospheric pressure plasma sources, main reactive components comprise of reactive neutral species (reactive oxygen and nitrogen species), UV-radiation, and electric current. In some cases charged species are present in sufficient amount and electromagnetic fields may play a role. DNA inactivation is not mainly caused by thermal effects, UV radiation, or electrical fields and charged particles, respectively, but by mainly plasma-generated reactive oxygen species.

Reactive oxygen species (ROS) are well known to play an important role in several biological systems. However, the production of ROS exceeding the ability of the organism to mount an antioxidant defense results in oxidative stress. If the amount of oxidative damage overcomes the repair capacity of a cell, this can ultimately lead to cell death, which is very important to be taken into account for biomedical applications of plasmas.

In previous studies the formations of SSBs and DSBs in dehydrogenated DNA molecules induced by the APPJ were studied with the main focus being on the contribution of particular plasma species to the total DNA damage [10]. The major cause of the DNA damage observed was found to be due to neutral metastable species, e.g., O_2 and He and radicals e.g., O and OH. Studies conducted by other research groups also have shown that when DNA molecules are exposed to plasma radiation strand breaks occur [11].

In our method, Gel electrophoresis has been used to check DNA form. After plasma treatment DNA samples were re-suspended in high purity water and then loaded into a 0.8% agarose gel (0.8 g of agarose dissolved in 100 ml of 1_ TAE buffer, which contains trizma base, acetic acid and ethylenediamine tetraacetic acid), prestained with SYBR green I dye[4]. We also investigated bacterial mutation by transferring of plasma treated DNA into bacterial cells. In transformation, the gene transfer technique is electroporation. Electroporation is a dynamic phenomenon that depends on the local transmembrane voltage at each point on the cell membrane [6].

1.3 Research Objectives

1.3.1 To investigate conditional parameters of the cold atmospheric pressure plasma jet.

1.3.2 To investigate effect of cold atmospheric pressure plasma on DNA conformation change.

1.3.3 To investigate effect of cold atmospheric pressure plasma treatment of DNA on mutation of bacterial cells which are transferred with the plasma-treated DNA.

1.4 Usefulness of the Research (Theoretical and/or Applied)

The research will provide understanding of biological effects and mechanisms of cold atmospheric pressure plasma interaction with living cells and DNA. This understanding will be significant in filling a blank of our knowledge in the safety issue of using the atmospheric pressure plasma jets and in the aspect of the low-energy plasma-DNA interaction. The research results will provide users of the atmospheric pressure plasma jets for biomedical treatments with updated information and warning on potential biological hazards of using the devices for applications in such as biomedicine, dentistry, skin beauty and agriculture. But on the other hand, the research may lead to finding of safety operation conditions of the atmospheric plasma jets for biomedical applications, also very instructive to the users.

1.5 Methodology and Scope

1.5.1 Measurement of the cold atmospheric pressure plasma jet machine including measurement of plasma power and plasma species (ROS, RNS) and designing of experiments and preparation of naked DNA.

1.5.2 Treatment of naked DNA by using the cold atmospheric pressure plasma jet and analysis of the treated DNA forms by using gel electrophoresis.

1.5.3 Transfer naked DNA treated by cold atmospheric pressure plasma jet into bacterial cells for observing mutation by using electroporation.