Treatment of rat wounds using plasma-jet parameters (Exposure time and gas flow rate)

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ABSTRACT
Worldwide, an estimated millions of patients seek medical care for wounds annually. Different methods and treatment materials have been examined and developed to promote wound healing. Recently, new technology, plasma medicine, has emerged to offer new solutions in wound care. To investigate the efficacy of non-thermal atmospheric pressure plasma jet (NAPPJ) for the treatment of wounds, they were performed 1 cm long and 0.5 cm wide using a surgical scalpel. Treatment was done immediately after the wound operation using an argon plasma jet with different gas flow rates (2.5, 5, 7.5, 10 and 12.5 slm) and exposure time (3, 6, 9 and 12 min). The treatment was done in a second day. After treatment done the wounds left for one day and in the third day, rats skin tissue has taken and tissue sections had made. The results showed that the mechanical strength of the wound repaired in the plasma treatment group was significantly higher than that in the control group. In addition, evidence from histological studies suggests that wound epithelium is significantly accelerated compared to the control group; vascular and fibrosis (collagen synthesis) increased significantly and the inflammatory stage in wound healing was shorter in the plasma treatment group. The percentage of the necrotic area was higher in the control group than in the plasma treatment group. Furthermore, exposure to voids within the NAPPJ resulted in a significant reduction in the number of neutrophils infiltrated. NAPPJ exposure to interstitials has a positive effect on wounds that lead to wound healing by limiting the progression of the injury. Also, the results show that the speed of gas flow increases the wound healing process and the speed of gas flow increases the active species and accelerates the healing process.

INTRODUCTION
Wound care and healing consider an important issues in health care. A long period of wound healing may cause patient discomfort, increase medical costs, and can endanger people’s lives. In order to improve wound healing, “plasma” is a common instrument with its ability to utilize the patient’s wound healing (Haertel et al., 2014). Blood clotting is an important issue in medicine, especially in the treatment of wounds. In last years, plasma has been used to increase the speed of blood clotting without any thermal effect, or damage to the surrounding living tissues. For example, in the treatment of wounds we need to form a blood clot to close the wound and
Figure 1: a) Jet of Argon plasma needle. B) Rat skin under a jet of Argon plasma needle.

at the same time to sterilize the surface that surrounding the wound (Srinivasan and Shobha, 2008). Plasma is defined as an ionized gas consisting of electrons, ions and electrically neutral particles of atoms or molecules (Alami, 2005; Tum and ., 2016). Langmuir is the first one who call the state of "ionized gas" by plasma, which is a Greek word that originally means "Formable material" or "gel" (Goldston and Rutherford, 1995; Fitzpatrick, 2006).

Plasma is also known as the fourth state of substance (Arora, 2013). Plasma makes up or form 99% of the universe (Dijk, 2012). The temperature of electrons is usually 10⁴ K, while the temperature of both neutral particles and ions is highly dependent on the type of produced plasma. Their temperature can vary or alter from room temperature to 10⁷ K. The sun is a common example of existing plasma in nature. Each plasma component usually has its own temperature, which means that for electrons Te, for Ti, and for neutral particles Tn. So it can be said that plasma is the only substance that has several temperatures at the same time (Kenda, 2007).

Recent trends concentrate on the use of plasma in the sterilization of medical equipment and treatment of living tissues. Where the main objective of tissue treatment by plasma is surgery does not cause any damage and high accuracy in the removal of diseased areas of the organism with minimal damage. Furthermore, the plasma has the ability to kill bacteria efficiently and quickly, which making it suitable for sterilizing surgical instruments and local sterilizing for tissues (Kieft, 2005). In 2006-2005, the Food and Drug Organization of the United States and the European Markets agreed that cold plasma or non –thermal plasma used as a treatment for skin restoration (plasma skin regeneration. PSR). Cold plasma restores damaged skin tissue (Bogle et al., 2007; Stoffels et al., 2006).

In 2010, Sameer proved that when utilizing an electrical insulation barrier discharge system, cold plasma interaction with living cells depends on the amount of dose to which these cells are exposed, which results in different types of oxygen reaction, which stimulates different effects. These include increasing the range of apoptosis, which depends on their concentration. Prefer to use cold plasma in the healing of wounds and the treatment of cancer (Ulhas, 2010).

In 2015 Cheng and his group made a small plasma-generating device and then obtained active plasma types and transported them to small targets such as a cancer cell. The distance between the tube of the plasma jet and the target is constant (Cheng et al., 2015).

In 2016, Ilik and Akan used the APPJ device with highly purified helium gas to generate cold plasma. The results showed that the plasma column length increases with the gas flow rate, and the density of the reactive species increases with increasing of gas flow rate (Ilik and Akan, 2016).

MATERIALS AND METHODS

Plasma classified to thermal and non – thermal plasma

Thermal Plasma
Figure 2: Measuring the temperature during treatment.

Figure 3: Treatment of wound surrounding area colored with Hematoxylin and eosin stain and the strength of zooming is (10 X).

Plasma in which electrons, ions and neutral particles (molecules or atoms) are at the same temperature. This means that it is in an equilibrium state with each other. Plasma in which electrons, ions and neutral particles (molecules or atoms) are at the same temperature. This means that it is in an equilibrium state with each other. Plasma is indicated as hot when fully ionized, in which $10,000K \approx Tn = Ti = Te$ (Ilik and Akan, 2016; , 1996).

Non-Thermal Plasma

Plasma is where neutral ions and particles are at the same temperature, while the temperature of electrons is much higher. Plasma is sometimes referred to as cold if the ionization is small 1% of the gas in which $10,000-100,000K \approx Te$ and $300-1000K \approx Tn = Ti$ (Tendero et al., 2006; Humud and Mahmood, 2013).

Active species

The active types is known RONS (reactive oxygen and nitrogen species) give a significant impact in the disable of bacteria. These species have obvious effects on cells through their interactions with various biomolecules. This will lead to damage the wall of the pathogenic bacterium and then leak the cytoplasmic content. It ultimately causes the death and disables of the bacteria, which are the affecting of chemical agents (Imlay, 2003). Since the jet of Argon plasma has a high concentration of active species (RONS) where the intensity of (OH radical) and has a direct impact on the cell wall or cell membrane of bacteria, such as atomic oxygen causes high oxidation, which leads to fat oxidation and disable of proteins and deterioration of DNA and thus damage of bacterial cell that cause wound inflammation (Mai-Prochnow et al., 2014).

The animals

A group of laboratory rats were used in this experiment and all animal conservation processes and procedures were compatible with the recommendations set by the Animals Ethics Committee affiliated to Biotechnology Research Center at Al - Nahrain University in Baghdad, Iraq. The rats were separated into a control group and an experimental group before work began.

Work method

The rat was anesthetized before the beginning of work. It anesthetized using xylazine, Ketamine and Halothane in order to prepare for work. Then the hair was removed and a wound was made 1 cm and a width of 0.5 cm using a surgical scalpel. The treat
Figure 5: Tissues sections for damage rat skin and doesn’t treatment. Also, it colored with Hematoxylin and eosin stain and the strength of zooming is (10 X).

Figure 6: The impact of plasma time in healing of wounds of rat skin which colored with Hematoxylin and eosin stain and the strength of zooming is (10 X).
Figure 7: The impact of plasma in wounds healing of rat skin with steady treatment time and changing the gas flow rate. The tissues colored with Hematoxylinand eosin stain and the strength of zooming is (10 X).
ment was done instantly after the wound process using an argon plasma needle. The treatment was once a day and the duration of treatment was one day, then it left for one day and the tissue was cut in the next day. The tissue sections had processed for Hematoxylin and eosin stain by using the standard protocol. After being placed on the slides, they were sent to the Iraqi Biotechnology Company in Baghdad, where the tissue had read there.

**Methods of wounds treatment using a jet of Argon plasma**

The plasma has effective in sterilizing wounds, killing bacteria and activating cells. The jet of Argon Plasma was used to treat wounds to a group of laboratory animals (rat). Argon gas used in these methods to produce plasma, so plasma needle used in the treatment of rat wound after made wound immediately as seen. In Figure 1. The treatment used twice a day. After treatment done the wounds left for one day and in the third day, rat skin tissue has taken and tissue sections had made. The current the study includes the groups as below,

Before starting work, the temperature should be measured during treatment by plasma. In spite of the temperature of the plasma is equal or reach to room temperature but may be increased during treatment using a plasma needle. This temperature is resulting of many factors like lab temperature, the distance, speed of gas flow and other factors lead to an increase the temperature. Therefore it will lead to a delay in the healing process. Measuring of plasma temperature during treatment is an essential matter, so the scale as a piece of a wound are a deal. Because the temperature is impact or effect on the cells and causes death, it have to be observed as shown in Figure 2. So it will effect on the healing process. Essentially, plasma temperature should be 40°C or less in the treatment process.

Figure 3 shows keep moving of plasma needle during treatment not only on the wound area but on surrounding areas to accelerate of the healing process.

**RESULTS AND DISCUSSION**

The studied animals are divided to four groups. Each group contains 20 rats as following.

The first group represents the healthy rats which are not exposed to any damage or wound in the skin, as it is shown in Figure 4. It is a tissue section for healthy rat skin colored with Hematoxylin and eosin stain and the strength of zooming is (10 X).

The second group includes the animals, which not treatment wounds, as shown in Figure 5. This figure shows tissues sections for damage rat skin and doesn’t colored with Hematoxylin and eosin stain and the strength of zooming is (10 X).

The third group includes wounds treatment by a jet of Argon plasma with a steady rate of gas flow at 2.5 slm, with changing treatment time (3, 6, 9 and 12 min). It had been used for different periods for about three minutes for each period. The results show that the healing process relatively related with treatment time, as shown in Figure 6.

The fourth group includes wounds treatment using a jet of Argon plasma with a steady time of treatment (2min) with changing gas flow rate at (2.5, 5, 7.5,10 and 12.5slm). The results show that the speed of gas flow accelerates the wound healing process, as shown in Figure 7.

**CONCLUSIONS**

Our results show that applying of cold plasma on a wound will accelerate the healing process. Cold plasma has the ability in clotting blood directly. In this research, the wound had exposed to cold plasma after made the wound directly. We have observed the tissues of the wound. The results show that the healing process relatively related with treatment time. The healing duration depends on increasing of the used dose. It means that more treatment time will lead to accelerating the healing process. Also, the results show that the speed of gas flow increases of the wound healing process. The speed of gas flow increases of active species and accelerate the healing process.

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