ORIGINAL ARTICLE

Investigation of Atmospheric Pressure Plasma Jet Effects on the Treatment of Glioblastoma Using PET Imaging

Mahdieh Bakhtiari Ramazani [1], Effat Yahaghi [2*], Morteza Nohekhan [1]

[1] *Plasma and Fusion Research School, Nuclear Science and Technology Research Institute, Tehran, Iran*

[2] *Department of Physics, Imam Khomeini International University, Qazvin, Iran*

*Corresponding Author: Effat Yahaghi
Email: yahaghi@sci.ikiu.ac.ir

Received: 27 August 2021 / Accepted: 01 December 2021

Abstract

Purpose: Cold Atmospheric Plasma (CAP) has a wide application in medicine that has many biological effects on bacterial, fungal, yeast, and mammalian cells, particularly cancer cells. In this study, the in-vivo anti-tumor effect of an Atmospheric Pressure Jet Plasma (APJP) source is investigated.

Materials and Methods: The Glioblastoma Multiforme (GBM) tumoric cells have been implanted in the rat brains. Then, cancerian cells are treated by direct CAP and the Temozolomide (TMZ) drug. Positron Emission Tomography (PET) imaging method is used for evaluating the tumor regions.

Results: The PET imaging results showed that the GBM cell has a significant reduction in direct plasma treatment and TMZ drug just. This reduction can be clearly observed just five days after treatment. The calculated Brain to the Background Ratio (BBR) shows that the cancer cells in the plasma method are halved after five days. This amount is comparable to the TMZ method that makes two times decreasing in the cancer cells.

Conclusion: The plasma method can be used as an effective method in treating and removing the GBM cells. Also, CAP has fewer side effects than chemical methods.

Keywords: Cold Atmospheric Plasma; Positron Emission Tomography Imaging; Glioblastoma Multiforme; Anticancer Effects; Atmospheric Pressure Jet Plasma.

DOI: https://doi.org/10.18502/fbt.v9i3.9649

1. Introduction

Glioma tumors originate in the glial cells of the brain and make up 30% of all central nervous system tumors and 80% of malignant brain tumors [1-2]. This set of cancerian cells includes tumors of glioblastoma, astrocytoma, oligodendroglioma, and ependymoma that have a high growth rate. Glioblastoma Multiforme (GBM), also referred to as a grade IV astrocytoma is the most common and aggressive type of brain cancer. GBM occurs most often in the cerebral hemispheres, especially in the frontal and temporal lobes of the brain. GBM cancer has a very high mortality rate and causes the death of the patient in less than 6 months. The signs and symptoms of GBM vary depending on the location of the brain tumor and may include headaches, double or blurred vision, speech difficulty of gradual onset, vomiting, loss of appetite, changes in mood and personality, changes in the ability to think and learn and new onset of seizures [3-5].

The main treatment for GBMs is including surgery, followed by radiation and chemotherapy. In the surgery, the tumor has been removed as much as possible and without injuring the normal brain tissue [1]. After surgery, radiation therapy can be used for killing the remaining tumor cells. In standard external beam radiation therapy, multiple sessions of standard-dose "fractions" of radiation are exposed to the tumor region in order to treat the tumor cells [3]. Each treatment induces damage to both healthy and normal tissue. The one important aim of radiotherapy is to have fewer effects on normal tissues. Also, chemotherapy with temozolomide is the current standard of treatment for GBM. This drug is generally described every day during radiation therapy and then for six cycles after radiation during the maintenance phase.

One of the newest and promising approaches to cancer therapy is Cold Atmospheric Pressure plasma (CAP). CAP or Non-Thermal Plasmas (NTP) are provided by inducing an electric or electromagnetic field to a gas. CAP is constituted basically by molecules and atoms in an excited state, positive and negative ions, free radicals, electrons, Ultraviolet (UV) radiation, and reactive oxygen and nitrogen species, such as ozone, superoxide, hydroxyl radicals, singlet oxygen, atomic oxygen, nitric oxide or nitrogen dioxide. Interestingly, all these agents show antimicrobial activity against a wide range of microorganisms, including bacteria, molds, yeasts, and even bacterial and fungal spores [3, 6-9]. Cold plasmas, with temperatures close to ambient temperature, are on the contrary suitable for the treatment of cancer cells. The various type of cold plasma can be produced with changing of the radio frequency, microwave frequencies, and different Alternating Current (AC) or Direct Current (DC) high voltages [10-12]. Today, various types of CAP sources have been developed at atmospheric pressure that are used in medicine and treatment. Several studies showed the significant anticancer capacity of CAP in over head and neck cancer, skin cancer, colorectal cancer, lung cancer, breast cancer, as well as bladder cancer [11-14].

Different methods are used to evaluate the efficacy of treatment in cancer such as evaluation of cells characters such as apoptosis and cell cycle by flow cytometry and imaging base method such as Computed Tomography (CT), Magnetic Resonance Imaging (MRI), and Positron Emission Tomography (PET). PET with the glucose analog [^{18}F]-Fluorodeoxyglucose (^{18}F-FDG) is a routine clinical test for most solid tumors [15]. In PET, positron-emitting radionuclides have been injected into the body. Radionuclide distributes in the body and positron will be emitted. Then, positron annihilates on contact with electrons after traveling a short distance (~2 mm). Each annihilation produces two 511-keV photons in opposite trajectories. These two photons are detected by the detectors. After collecting data from the desired region of the body, the data is processed by computers to reconstruct the spatial distribution of the radiotracers. The PET images have the ability to visualize, characterize, and measure biological processes at the cellular, subcellular, and molecular levels in living subjects using noninvasive procedures. PET can also measure biochemical and physiological aberrations that occur prior to macroscopic anatomical signs of a disease, such as cancer [15]. In some research, the beneficial role of cold physical plasma, treatment outcomes, potential risks, and underlying mechanisms are investigated by the PET imaging method [16-18]. In this study, the anti-cancer effect of Atmospheric Pressure Jet Plasma (APJP) is investigated using helium gas on GBM cancer cells. The cells were grown in the anterior part of the rat brain. The results were surveyed by the PET method.

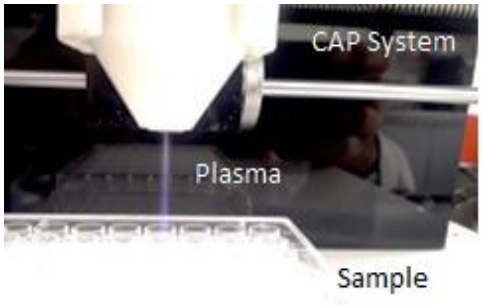
2. Materials and Methods

2.1 Design and Manufacture of Atmospheric Pressure Jet Plasma (APJP)

Figure 1a shows the plasma jet device that was used as the CAP system in this research. The atmospheric

pressure jet plasma consists of a dielectric tube with two metal ring electrodes, which are located on the outside of the dielectric tube. When a working gas such as helium flows through a dielectric tube and an AC (the kHz range) high voltage is applied, a cold plasma jet is produced in the surrounding air. The system is fed with an AC power supply with a sinusoidal wave and high voltage of 5kVolt/20 kHz. In this plasma jet, a helium gas flow was used with a flow rate of 2 lit/min. The flow rate is controlled by Mass Flow Meter, and a 2 mm diameter gas hole for open helium was used for system operation. Figure 1b, shows the plasma jet interacting with U-87 MG cells.

a



b

Figure 1. a) the plasma jet system, b) Photograph of the plasma jet interacting with U-87 MG cells

2.2 Cell Growth Protocol for U-87 MG Cells

The U-87 MG cells were purchased from Pasteur Institute Cell Bank (Tehran, Iran) and cultured in Dulbecco's Modified Eagle Medium (DMEM) / F12 medium (Sigma-Aldrich Corp. St.Louis, MO, USA). The used media were enriched with 10% Fetal Bovine Serum (FBS) and antibiotics penicillin and streptomycin (1% pen / strep) (Gibco, Thermo Fisher Scientific Inc, Rockford, USA). The cells were stored at 37 °C in 5% CO_2 at 95-90% humidity and subjected to successive passages using trypsin (Gibco, Thermo Fisher Scientific Inc, Rockford, USA) and Phosphate-Buffered Saline (PBS) [4].

The MTT assay (MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) is used to measure cellular cytotoxicity and cell viability. To prepare the MTT solution, 5 mg of MTT powder is dissolved in 1 ml of PBS solution. After filtering of this solution, it is stored at -20 °C. This solution should not be exposed to light, so the storage tubes were covered with foil. One ml of MTT solution is added to the cultured cancer cells and then the cancer cell is irradiated by plasma in different time intervals between 30 to 90 seconds. After irradiating, the viability of the cells is assessed by the MTT method after 24, 48 and 72 hours (Figure 2). The percentage of cell survival is calculated by the following formula, Equation 1:

$$\textit{The survival percentage of each treatment sample} = \frac{\textit{treatment sample uptake}}{\textit{control sample upake}}) \textit{ x } 100\% \quad (1)$$

The IC_{50} values (that is, the plasma exposure times which exhibited 50% cell viability for U-87 MG cells) were 98.91, 70.7, and 34.37 s, respectively, for 24, 48, and 72h after treatment. The results show that the best treatment time is 70.7 s for IC_{50}.

2.3 GBM Cancer Implantation and Treatment with CAP and Temozolomide

In this research, 24 rats have been chosen for in-vivo tests. The chosen rats weighing 200 to 250 g were

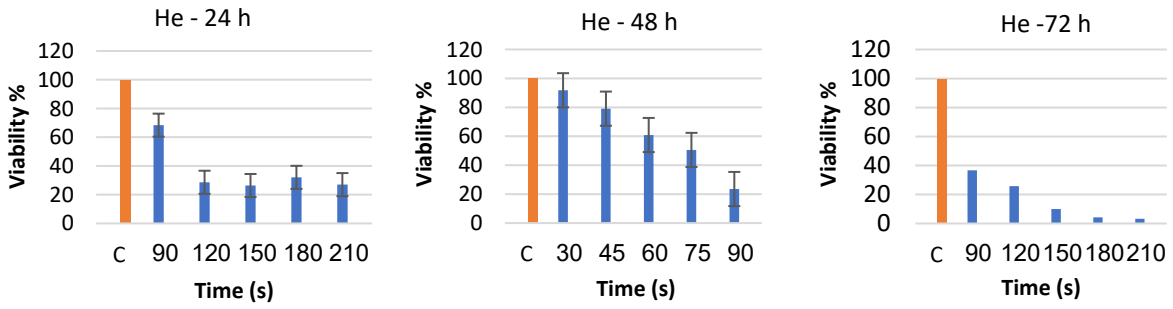


Figure 2. Viability of the U-87 MG cells a) 24 h, b) 48 h, and c) 72 h after treatment

anesthetized by the peritoneum injection of ketamine /xylazine (relative to 100 mg/kg body weight / 20 mg/kg body weight) and kept in a stereotaxic frame. Two holes were made in the skull above the right hippocampus in a distance of 2.2 mm of the Anterior-Posterior (AP), 1.9-mm of Medial-Lateral (ML), and a depth of 2 mm from the Abdomen. The cancer cells were injected into the holes. Fifteen days after tumor GBM cancer implantation in rats, plasma radiation was directly exposed to the tumor region through a channel that was embedded in the rat brains. The plasma jet has been applied in two sequential days for 70.7 seconds for the first group. Figure 3 shows the procedure of the plasma injection into the cancer region in the rat head. The Temozolomide (TMZ) drug 0.01 g/l was also given to the second group for treatment. TMZ is a medication used to treat some brain tumors such as GBM or anaplastic astrocytoma. It is taken by mouth or via intravenous infusion. The rats were sent to the laboratory for PET imaging one, two, and five days after treatments.

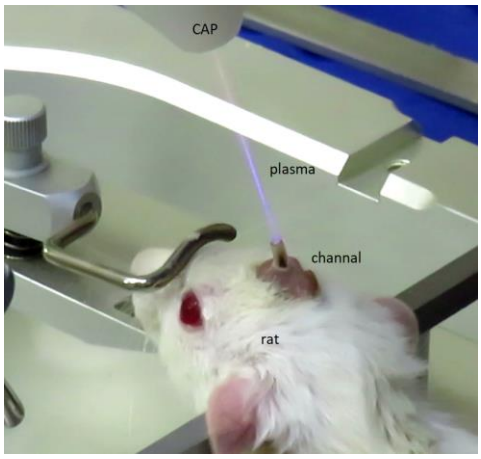


Figure 3. Shows the procedure of the plasma injection to the cancer region in the rat head

2.4 PET Images

PET with 2-^{18}F-FDG [15] has been performed on un-treated rats. Besides, PET imaging has been carried out for treated rats 1 day and three days after treatment for the evaluation of the direct APJP treatments. The PET images were obtained with a micro-PET scanner (Xtrim PET, Parto Negar Persia, Iran) in the preclinical core facility lab of Tehran University of Medical Science. For PET scanning, about 1 mCi of the ^{18}F-FDG was injected via the tail vein of the rats under general anesthesia. For each rat, 3-dimensional Regions Of Interest (ROIs) were manually drawn over the brain area transversal images. The ROIs was converted to the Brain to Background Ratio (BBR) according to Equation 2:

$$BBR = \frac{ROI\ counts\ per\ voxel}{ackground\ counts\ per\ voxel} \quad (2)$$

3. Results and Discussion

After GBM tumor cell injection and over the 15 days, tumors were detectable by PET imaging (Figure 4). The rats were divided into three groups, two groups treated by direct CAP and the TMZ drug, while no treatment was performed on the third group (control group). CAP treatment was started on 15 days four after GBM tumor implantation and followed for two days for 70.7 seconds according to IC_{50} and MTT experiments which were described in section 2.3. To assess the implemented treatment methods, the effects of CAP therapy on GBM tumor cell were evaluated by PET images.

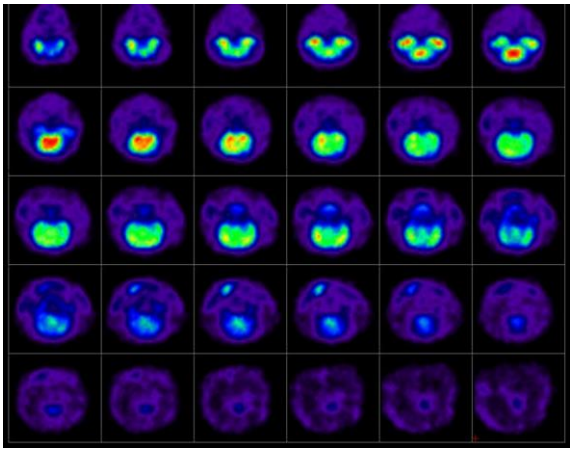


Figure 4. Axial slices for an untreated mouse

Figure 5 shows the axial slices of the PET images for a treated rat one day after treatment. Different regions of the brain of rats have various uptakes. The most uptakes have occurred at the tumor region (red color). Figure 5A has been generated by superimposing the images of the first day after treatment. The eyes of rats on both sides of the head and the tumor region (below of it) have the most uptakes. Also, the red color shows the high density of cancer cells in the image. Figures 5B and C show the superimposed images of PET, 2 days and 5 days after treatment, respectively. A comparison of the images of Figure 3 shows that the tumor region after two days of the treatment with CAP is reduced (Figure 5B). In addition, the tumor is almost removed after five days of the treatment (Figure 5C).

For a quantitative evaluation, the uptake ratio is calculated as brain to background. BBR has been obtained for treating with CAP and the TMZ drug (Table 1). The results show that the calculated BBR factor for the treated rats is twice lower than the untreated ones. Reduction of cancer cells after treatment can be seen in cross-sectional and three-dimensional PET images for the treatment of GBM tumor by cold helium plasma jet. PET images revealed significant decreased tumor cells in the treated group over the observation time.

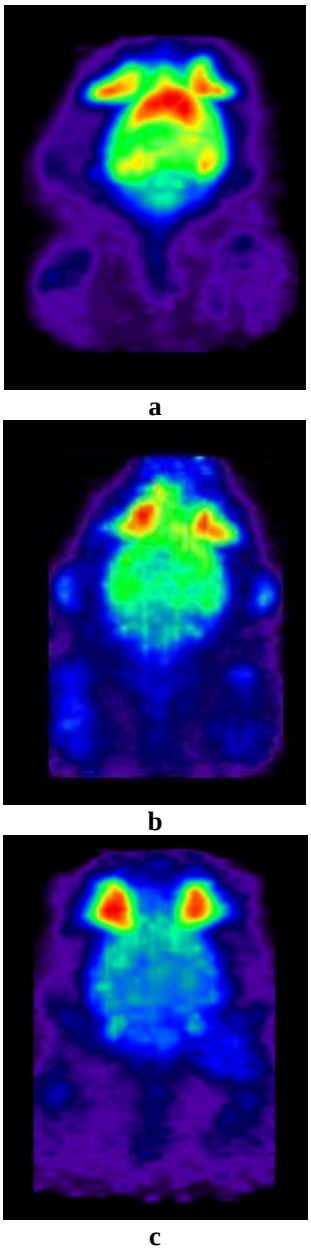


Figure 5. the superimposed images superimposed of PET images a) the first day of after treatment b) 2 days and c) 5 days after treatment, respectively

The results show that CAP therapy caused a significant reduction in anatomical tumor volume in five days, compared to the first day. Also, the comparison of the results of CAP method and the TMZ drug shows that although the amount of BBR in CAP is larger than TMZ on the first day after treatment, the amount of BBR by the two mentioned methods is slightly different in the five days after treatment. Nausea and vomiting, constipation, headache, and fatigue are mentioned as side effects (occurring in greater than 30%), for patients taking TMZ. Very rare side effects have been reported for plasma jet in different researches.

Table 1. The amount of the radionuclide uptake ratio of the selected ROIs in the 3 days PET scan of each rat

Time after treatment	BBR for treating with CAP	BBR for treating with TMZ
1 day after treatment	10.26	6.73
2 days after treatment	3.53	-
5 days after treatment	4.62	4.33

The discharged power for the plasma jet was set to a maximum power of 2 watts in this experiment. This level of power cannot cause inflammation in the tissue. On the other hand, noble gases such as helium and argon do not cause any inflammation in the tissue. One advantage of cold plasma is the apoptosis of cancer cells with a low level of ionization without any damage to healthy cells. Other treatments of cancer such as chemotherapy or radiotherapy cause inflammation in tissue and some damage to the healthy cells [19-21].

4. Conclusion

In this study, an APJP cold plasma system was designed and fabricated. It was used for direct cancer therapy. The therapeutic effects of the system were investigated for the direct method and also by using helium on GBM brain tumors of the rats. The best time duration for plasma treatment was determined using the MTT experiment and the IC_{50} factor by the in-vitro method. This time is obtained as 70.7 s that was used for the therapy of the rat tumor. The plasma and also TMZ drug were injected into different groups of the experiment and the effects of plasma on the cancer cell were investigated. The PET imaging system is used for evaluating the cancer region in the rat brains. As the main result, PET imaging revealed a substantial reduction in tumor cells after CAP treatment and TMZ drug for the GBM tumor. The amount of the BBR factor in both

groups of plasma and TMZ tumor for five successive days after the last treatment shows that the amount of BBR is slightly different in the two experimental groups. Due to the fewer side effects of plasma compared to the TMZ drug, plasma jet can be used as an effective and efficient method to treat the GBM cells. These findings propose APJP as a potential adjuvant therapy option to established standard therapies of GBM cancer.

Acknowledgments

We thank the preclinical facility of Tehran University of Medical Science for providing facilities for PET imaging.

References

1- V. Gilard, A. Tebani, I. Dabaj, A. Laquerrière, M. F. S. Derrey, S. Marret, and S. Bekri, "Diagnosis and Management of Glioblastoma: A Comprehensive Perspective.", *J. Pers. Med*., 11(4), 258, (2021). https://doi.org/10.3390/jpm11040258.

2- Zh. Chen, H. Simonyan, X. Cheng, E. Gjika, L. Lin, J. Canady, J. H. Sherman, C. Young, M. Keidar, "A Novel Micro Cold Atmospheric Plasma Device for Glioblastoma Both In Vitro and In Vivo.", *Cancers*, 9, 61, (2017); doi:10.3390/cancers9060061.

3- P. Attri, N. Kumar, J.H Park,. D.K. Yadav, S.Choi, H.S.Uhm,; I.T Kim.; E.H. Choi, W.Lee, "Influence of reactive species on the modification of biomolecules generated from the soft plasma.", *Sci. Rep*., 5, 08221, (2015).

4- S. B.Lyne, B.Yamini, "An Alternative Pipeline for Glioblastoma Therapeutics: A Systematic Review of Drug Repurposing in Glioblastoma.", *Cancers*, 13, 1953, (2021). https://doi.org/10.3390/cancers13081953.

5- P.B.Flynn; A. Busetti.; E. Wielogorska; O.P.Chevallier; C.T. Elliott, G. Laverty, S.P Gorman, W.G Graham.; B.F. Gilmore. "Non-thermal Plasma Exposure Rapidly Attenuates Bacterial AHL-Dependent Quorum Sensing and Virulence.", *Sci. Rep*., 6, 26320, (2016).

6- M. Yousfi, N. Merbahi, A. Pathak, O.Eichwald, "Low-temperature plasmas at atmospheric pressure: Toward new pharmaceutical treatments in medicine.", *Fundam. Clin. Pharmacol*., 28, 123–135, (2014).

7- E. Robert, M. Vandamme.; L. Brullé.; S. Lerondel; A. le Pape, V. Sarron, D. Riès, T. Darny, S. Dozias, G. Collet, "Perspectives of endoscopic plasma applications." *Clin. Plasma Med.*, 1, 8–16, (2013).

8- Z. Chen, L. Lin, X. Cheng, E. Gjika, M. Keidar, "Effects of cold atmospheric plasma generated in deionized water in cell cancer therapy.", *Plasma Process. Polym*., 13, 1151–1156, (2016).

9- S. Kalghatgi, G. Friedman, A. Fridman, A.M. Clyne, "Endothelial cell proliferation is enhanced by low dose non-thermal plasma through fibroblast growth factor-2 release.", *Ann. Biomed. Eng*., 38, 748–757, (2010).

10- H.M. Joh, S.J. Kim, T. Chung, S. Leem, "Reactive oxygen species-related plasma effects on the apoptosis of human bladder cancer cells in atmospheric pressure pulsed plasma jets.", *Appl. Phys. Lett*., 101, 053703, (2012).

11- D.N. Cagney, A.M. Martin, P.J. Catalano, A.J. Redig, N.U.Lin, E.Q. Lee, P.Y Wen, I.F. Bi W.L Dunn, S.E Weiss, et al, "Incidence and prognosis of patients with brain metastases at diagnosis of systemic malignancy: A population-based study.", *Neuro-Oncology*, 19, 1511–1521, (2017).

12- S. Reuter, T. Von Woedtke, K.-D. Weltmann, "The kINPen—A review on physics and chemistry of the atmospheric pressure plasma jet and its applications.", *J. Phys. D Appl. Phys.*, 51, 233001, (2018).

13- C. Tendero, C. Tixier, P. Tristant, Desmaison, J.; Leprince, P. "Atmospheric pressure plasmas: A review. Spectrochim.", *Acta Part. BAt. Spectrosc*., 61, 2–30, (2006).

14- D. Yan, J.H. Sherman, M. Keidar, "Cold atmospheric plasma, a novel promising anti-cancer treatment modality.", *Oncotarget*, 8, 15977–15995, (2016).

15- M. Kordt, I. Trautmann, Ch. Schlie , T. Lindner, J. Stenzel, A. Schildt, , Boeckmann, L., Bekeschus, S., Kurth, J., Krause B. J., Vollmar, Grambow E., "Multimodal Imaging Techniques to Evaluate the Anticancer Effect of Cold Atmospheric Pressure Plasma.", *Cancers*, 13, 2483, (2021). https://doi.org/10.3390/cancers13102483.

16- A. Vaid, C. Patil, A. Sanghariyat, R. Rane, A. Visani, S. Mukherjee, A. Joseph, M. Ranjan, S. Augustine, K. P. Sooraj, V. Rathore, S. K. Nema, A. Agraj, G. Garg, A. Sharma, M. Sharma, K. Pansare, C. M. Krishna, J. Banerjee, S. Chandra. "Emerging Advanced Technologies Developed by IPR for Bio Medical Applications. A Review.", *Neurol India*, 68:26-34, (2020)

17- A. D. Bonzanini, K. Shao, A. Stancampiano, D. B. Graves, and A. Mesbah, "Perspectives on Machine Learning-assisted Plasma Medicine: Towards Automated Plasma Treatment.", *IEEE Transactions on Radiation and Plasma Medical Sciences*, (2021), DOI: 10.1109/TRPMS.2021.3055727

18- A. Schmidt, Th. V. Woedtke, J. Stenzel, T. Lindner, S. Polei, B. Vollmar and S. Bekeschus, "One Year Follow-Up Risk Assessment in SKH-1 Mice and Wounds Treated with an Argon Plasma Jet.", *Int J Mol Sci*. 19;18(4):868, (2017). DOI: 10.3390/ijms18040868

19- Y. Park, H. J.Kim, J. Y. Lee, S. H Jeong, E. Y. Kwon, J. Y. Joo, "Anti-inflammatory effect of non-thermal atmospheric pressure plasma for periodontitis treatment: in vitro pilot study.", *Journal of Dental Rehabilitation and Applied Science*, , 37(2), 88-94, (2021).

20- S. Bekeschus, J.Moritz, I. Helfrich, L. Boeckmann, K. D. Weltmann, S. Emmert, T. Von Woedtke, "Ex vivo exposure of human melanoma tissue to cold physical plasma elicits apoptosis and modulates inflammation.", *Applied Sciences*, , 10(6), 1971, (2020).

21- H. R. Metelmann, C. Seebauer, V. Miller, A. Fridman, G. Bauer, D. B. Graves, T. Von Woedtke, "Clinical experience with

cold plasma in the treatment of locally advanced head and neck cancer.", *Clinical Plasma Medicine*, 9, 6-13, (2018).