Plasma Medicine, 12(2):1–14 (2022)

Characterization and Assessment of Cold Plasma for Cancer Treatment

Aavash Shakya,[a] Suman Prakash Pradhan,[b] Niroj Basnet,[a] Rajendra Shrestha,[c,d] Pusp Raj Joshi,[e] Roshan Gautam,[f] Aakash Paneru,[g] Ashok GC,[g] Arun Kumar Shah,[d] Rameshwar Adhikari,[h] Deepak Prasad Subedi,[a] & Sagar Regmi,[a,h,i,j,*]

[a]Department of Physics, School of Science, Kathmandu University, Dhulikhel, Nepal; [b]Department of Environmental Science and Engineering, School of Science, Kathmandu University, Dhulikhel, Nepal; [c]Department of Physics, Nepal Banepa Polytechnic Institute, Banepa, Kavre, Nepal; [d]Department of Physics, Patan Multiple Campus, Tribhuvan University, Lalitpur, Nepal; [e]Annapurna Research Center, Kathmandu, Nepal; [f]School of Computing and Digital Technology, Birmingham City University, United Kingdom; [g]Department of Chemical Science and Engineering, School of Engineering Kathmandu University, Dhulikhel, Nepal; [h]Research Centre for Applied Science and Technology, Tribhuvan University, Kathmandu, Nepal; [i]Nepal Academy of Science and Technology, Lalitpur, Nepal; [j]Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA

*Address all correspondence to: Sagar Regmi, Case Western Reserve University, School of Medicine, Cleveland, OH 44106, USA; Tel.: +1(216)-808-5879, E-mail: sagar005@e.ntu.edu.sg

ABSTRACT: The unbalanced lifestyle and rampant use of modern medicine are the leading causes of life-threatening cancer disease prevalence. In the last few decades, chemotherapy and other medication techniques promised to cure cancer. However, cold plasma on cancer cell lines gets little attention due to a lack of understanding of the detailed mechanism of action. In the contemporary time frame, it is well established that cold plasma therapy is one of the best alternatives for treating cancer. The selectivity of cancer cells by plasma treatment has a unique potential as therapeutics in future clinical practices. In this study, we analyzed the potential of cold atmospheric plasma (CAP) irradiated medium as a promising anti-cancer tool by using a high-voltage power source with a 20 kHz operating frequency. The discharge was generated with argon as working gas and characterized by optical emission spectroscopy. Eagle's minimum essential medium (EMEM) was treated with CAP utilizing argon as a plasma source at 2–3 kV for varied time durations (0 min, 1 min, 2 min, 3 min, 4 min, and 5 min) to demonstrate the anti-cancer capabilities of CAP treated media. The treated media culture grows cervical cancer cells (HeLa), breast cancer cells (MCF-7), mouse fibroblast cell line (3T3), and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was carried out to determine the cell viability and inhibition rate. Our results revealed a considerable difference in viability between cancer cells and normal cells as treatment time increases from 1 to 5 min. The cell viability for HeLa (15.23%) and MCF-7 (16.18%) as compared to 3T3 cells (134.56%). This study provides evidence for the potential of CAP-treated media as an avenue for an anti-cancer representative.

KEY WORDS: anticancer, cold plasma, cell viability, cell inhibition, medium treatment

I. INTRODUCTION

Cancer is defined as the abnormal growth of cells that might originate from the evolution and division of cells, steady growth, evading programmed cell death, uncontrolled cell division, angiogenesis, and invasion of tissue, and creation of metastasis causes dysfunctionalities of normal cells within the human body. All tumor cells display these six hallmarks mentioned above of cancer, with these features producing a malignant tumor.[1] Cancer is one of the leading causes of death in the twenty-first century and the most severe impediment to increasing global life expectancy. There are about 200 types of cancer, often triggered through a histological level where the disease was discovered for the first time.[2] Breast cancer and cervical cancer are the two most frequent cancer worldwide. Despite significant advances in medical sciences and biology, developing new, practical, and inventive cancer treatment methods remain complicated, requiring diverse collaborations. Chemotherapy is well established traditional cancer therapy adapted globally. However, the severe side effects of chemotherapy make it urgent to search for alternative cancer treatment methods with high accuracy and economic viability.[3] So, cancer researchers are constantly trying to identify innovative and practical tools to lessen the severe side effects of traditional cancer treatments.

Ionizing different gases produce plasma into an electric field by decomposing various reactive species, sub-atomic particles, and neutral particles.[4] This electroneutral mixture of particles with quanta of electromagnetic radiation and strong electrical fields[5–7] makes plasma to be applicable in different fields such as material processing, biomedical, environmental remedies, food safety, water treatment, and so on.[8] Cold plasma; described by specific difference between the electron temperature and other species temperature, is generated under slight conditions of atmospheric temperature and pressure.[9] Cold plasmas can be used as practical tools for the scientific administration of various organisms ranging from microbes to human beings.[10–14] Moreover, the application of cold atmospheric plasma (CAP) in cancer treatment is a new area of interest in contemporary research, as it has a broad perspective in various biomedical applications such as treatment of dermatological disorders, cardiovascular disorders, dental problems, wound healing, and multiple types of cancer.[11–13,15,16] The scope of CAP for cancer therapy is rapidly mounting to address previously untreatable targets, such as those with metastatic potential and medication resistance. Integrated research on the multi-dimensional effect of CAP in cancer treatment is required to proceed toward a widespread clinical application of CAP.[17]

The science behind plasma cancer treatment and possible clinical implementation has been crucial for a better understanding of the application of plasma in cancer treatment. In this work, we analyzed the effectiveness of CAP in cancer treatment. Under mild conditions, simple devices such as corona discharges, glow discharges, dielectric barrier discharges, and plasma jets are widely used to generate plasmas with predefined characteristics.[14,18] Nevertheless, due to CAPs' unusual chemistry and significant reactivity, many chemical reactions can occur that would be difficult or impossible to achieve

using other standards methods.[14,19,20] Furthermore, numerous previous investigations on diverse uses of CAP revealed the rapid evolution and promising source for this new interdisciplinary division between physics and medical sciences. Despite several documentations of plasma disinfection and therapies, the mechanism of actions of plasma with biological belongings such as plant seeds, living cells, or microorganisms remains unknown to date.[21] In this study, we analyzed the application of CAP in potential use as an alternative for cancer cell treatment through *in vitro* MTT assay.

II. MATERIALS AND METHODS

A. Experimental Setup

The experiment was conducted using argon as a principal working gas. The detail of the experimental setup is shown in Fig. 1. The plasma jet setup consists of a two-electrode system; the lower electrode was constructed with a copper tape of 1 mm thickness wrapped around an outer part of the quartz tube (4 mm in diameter), and the upper electrode was made up of stainless steel with gas inlet fitted in the inner part of the quartz tube (3 mm in diameter). A high voltage power supply was connected to the upper electrode, and the lower electrode was grounded. The two electrodes were set at a distance of 12.5 mm, while the gap between the nozzle and sample was set at 20 mm. The gas

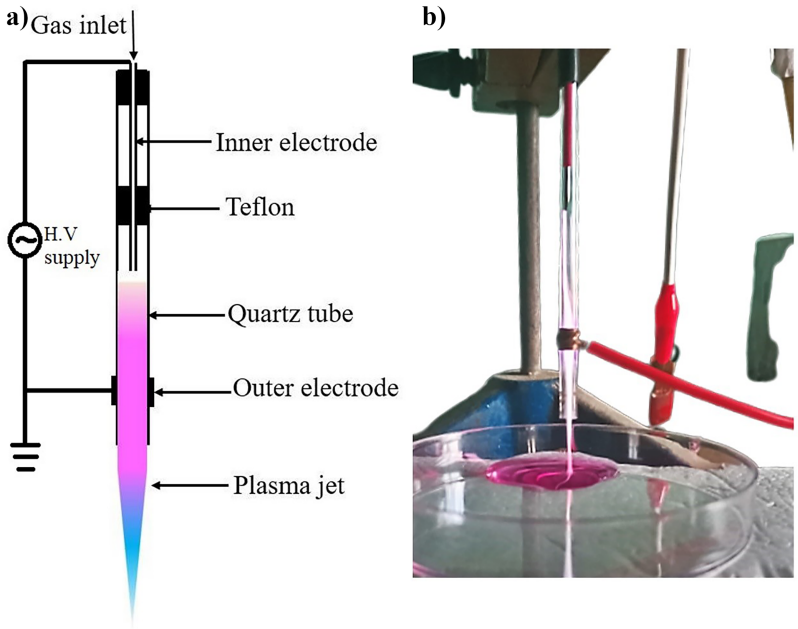

FIG. 1: (a) Schematic diagram and (b) working conditions of atmospheric pressure argon plasma jet

flow rate of argon was set at 2 liters/min. The applied voltage and frequency were 2 kV and 20 kHz, respectively.

B. Chemicals

Eagle's minimum essential medium (EMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), glutamine, penicillin, and streptomycin were purchased from Caisson Lab, USA. Dimethyl sulphoxide (DMSO) was purchased from Merk, Germany, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye was purchased from Himedia, India, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich, USA.

C. Cell Culture and Growth Conditions

To maximize the cell harvest, the cells were subjected to various steps to grow and reproduce correctly, including cell trypsinization, cryopreservation, and cell revival. In a water bath, the cryopreserved cells were thawed. The cell suspension was mixed with medium and centrifuged at 300 g for 4 min. Then, the suspension was combined with EMEM in a T-25 cell culture flask and incubated in a CO_2 incubator. Next day, the appropriate media was changed. At the sub-confluent stage, the cells were passaged. In a 15 mL centrifuge tube, the trypsinized cell suspension was collected. The cells were extracted by centrifuging them for 4 min at 300 g at 25°C.

D. Analysis of Cell Viability

The cell viability of analyzed cell lines against plasma treatment was determined using MTT (3-[4, 5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay with slight modification. HeLa, MCF-7, and 3T3 cells were grown in T-25 flasks with EMEM medium supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% L-glutamine. The culture was incubated at 37°C in a 5% CO_2 incubator.[22] Following 80% cell confluence and attachment, the number of cells grown for 48 hours in a 96 well cell culture plate was around 1.5×10^4 per well for all the cell lines before introducing plasma activated medium (PAM). The number of cells was calculated using a hemocytometer. The PAM was administered at different times (1, 2, 3, 4, and 5 min). After a 48-hours incubation, the supernatant was collected from each well, and 50 µL of 5 mg/mL MTT was added. A purple formazan product was generated after 4 hours of incubation. Around 100 µL of 2.5% DMSO (v/v) was used to dissolve the formazan crystals. At 595 nm, the absorbance was measured with a microplate reader (Azure Biosystems) as the best absorbance was found in that wavelength.[23,24] The following formula was used to compute the percentage of cell viability.

$$\text{Percentage of Cytotoxic Activity} = A_t/A_0 \times 100\%$$

where A_0 represents the absorbance of cells without PAM, and A_t represents the absorbance of cells with PAM.[25]

E. DPPH Free Radical Scavenging Assay

The free radical scavenging activity was assessed by DPPH assay.[26] In brief, 1 mL of plasma-treated media (1–5 min) mixed with 3 mL DPPH (100 μM) and incubated in the dark for 30 mins. Then, the reaction mixtures' absorbance was measured at 517 nm in UV-visible spectrophotometer (UV-Shimadzu, 1800).

F. Statistical Analysis

All data were analyzed triplicate and presented as mean ± standard deviation (mean ± SD) except for the variability calculation, where data are presented as mean ± standard errors (mean ± SE). Data were analyzed by one-way analysis of variance (ANOVA) using IBM SPSS version 24. All graphical representation were prepared by Origin 2018 and GraphPad Prism version 6.

III. RESULTS AND DISCUSSION

A. Characterization of Cold Plasma

An optical fiber visual analysis was used to assess our CAP device. At atmospheric conditions, the plasma jet discharge was made to flow through the optical fiber, and the spectra were captured by the Ocean Optics (USB 2000+). Figure 2 depicts the discharge spectra as a plot of the intensity wavelength at atmospheric pressure. The argon flow rate was 2 L/min. The electron temperature T_e is determined using the Boltzmann plot method given by Eq. (1).

Moreover, the discharge of plasma was characterized by the Boltzmann plot. A total of five suitable lines for Ar II were taken from the optical plot of spectral lines as shown in Fig. 2, which represents the optical spectra of the plasma jet taken from the optical spectrometer. Assuming a Boltzmann distribution exists, it is a linear plot. Thus, observing components in the right portion of Eq. 1 demonstrates that the slope (m) of the Boltzmann distribution plot represents plasma temperature. The formula to analyze the electron temperature (T_e) of the plasma was taken from previous studies.[27,28]

$$\ln\frac{I\lambda}{gA} = -\frac{1}{K_B T_e}E + C \tag{1}$$

The selected ArII line's corresponding energy levels and the statistical weight required for calculation are presented in Table 1. The values of A, g, and E were taken from the National Institute of Standards and Technology (NIST). E is the upper-level

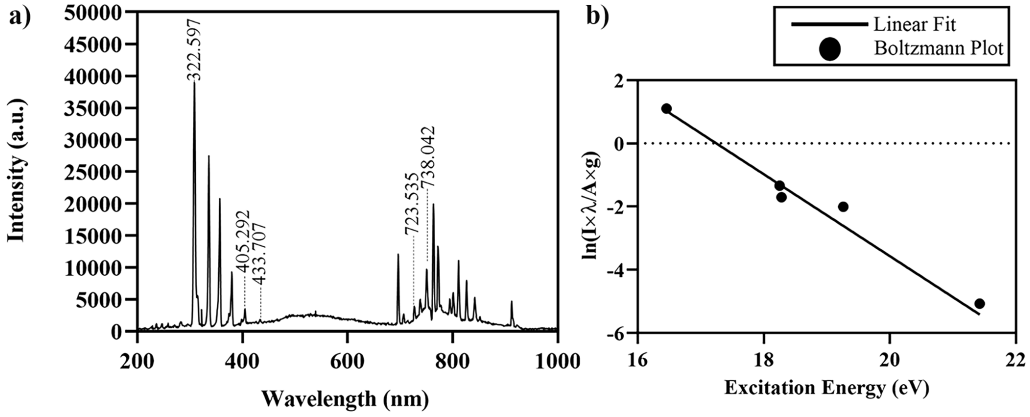

FIG. 2: (a) Optical emission spectra of CAP. (b) The plot between E and ln(Iλ/gA), the slope of the linear fit gives the value of $1/K_B T_e$ from which, we can obtain the electron temperature of the plasma. In this case, we obtain our electron temperature to be 0.79 eV, which is discharged with 2.4 kV voltage and 20 kHz applied frequency in an Argon environment (flow rate; 2 liters/min).

TABLE 1: Data of corresponding lines spectra from the NIST database[29]

Wavelength (λ)	Intensity (I)	A*g	Energy (eV)	ln(Iλ/gA)
433.707	2030.58	1.4e + 008	21.426	–5.06871
405.292	3849.74	2.7e + 008	20.74	–5.15356
322.597	3519.18	8400000	19.26	–2.00136
723.353	5438.4	1.5e + 007	18.25	–1.33842
738.042	5438.4	2.2e + 007	18.28	–1.70131

energy, K_B is the Boltzmann constant, and T_e is the electron temperature. The transition probability (A), statistical weight (g), and energy levels (E) for the argon II lines were obtained from the (NIST) atomic spectra database, and the transition probability, statistical weight, and energy levels for the argon II lines were derived from the observation. The temperature of electrons was calculated during argon discharge at atmospheric pressure, assuming that the local thermodynamic equilibrium (LTE) was achieved.[29]

The plot of the above equation with E on the horizontal axis and ln(Iλ/gA) on the vertical axis. The electron temperature T_e determine from the slope of the best fit line. As shown in Fig. 2, the electron temperature is 0.79 eV.

B. Anti-Cancer Potential

Cancer has been one of the leading causes of mortality. According to World Health Organization (WHO), it accounted for nearly 10 million deaths in 2020.[30] With the

progress of science and technology, there has been a significant improvement in cancer therapy and increased quality of life. Nonetheless, the high prevalence of different cancers, and each needs a different treatment strategy, makes it challenging to find a particular treatment. Conventional treatment (chemotherapy) has shown promising results, but its toxic nature comes with many sacrifices. Some cancer even develops resistance to this treatment method. Besides, the scientific community is working hard to find new and efficient alternative treatments to reduce these harmful side effects.[31] As cancer is unpredictable, holistic, and cost-effective, cancer therapy is critical in justifiable medical development.[32]

In this study, we analyzed the viability of two different cancer cell lines, namely, HeLa (Human cervical cancer cell line), MCF-7 (Human breast cancer cell line),[33] and 3T3 (Mouse fibroblast cell line) against the cold plasma treated (1–5 min) growth medium. The interaction and activities of cancer cell growth show the inverse relation with plasma treatment time. When the medium treatment period approaches 5 min, only 15.23% of HeLa and 16.18% of MCF-7 cells remain viable, but in the case of the mouse fibroblast cell (3T3), the CAP seems to increase the cell viability by 134.56%. Figure 3 depicts the percentage of cell viability of HeLa, MCF-7, and 3T3 cells *in vitro* as a function of treatment time using cold plasma technology. There was no significant difference in viability between cancer and mouse fibroblast cell lines when cells were grown in 1 min plasma-treated media. In the case of HeLa cells, there are no significant decreases in cancer cells for 1 min treated PAM. Similarly, the significant impacts of PAM was seen in MCF-7 cell growth. We observed the more cell death in increased treatment times of cancer cells. In addition, there is a significant difference in viability between cancer cells and mouse fibroblast cells when grown in PAM and treated for 2 to 5 min. The inhibition percentage of mouse fibroblast cells was negative 34%. While the inhibition of HeLa and MCF-7 were acquired up to 84% and 83%, respectively, for 5 min treatment time (Fig. 4). Our results revealed a significant change in viability impacts of plasma on cancerous and non-cancerous cell lines along with increasing treatment time. In the mouse fibroblast cell line, 3T3 cells are less susceptible to CAP treated medium than HeLa and MCF-7 cells at all treatment times. This signifies anti-proliferative impact on cells depended on the duration of CAP treatment of the EMEM medium.

Stoffels et al. in 2002 led to the use of plasma jet devices in biomedical applications. Their study found that cold plasma has no thermal damage on the surface of organic material.[34] Laroussi et al. and Lu et al. used helium gas as a carrier and found that it can be touched with a bare hand.[35–37] Furthermore, Fridman et al. used cold plasma to promote blood coagulation and tissue sterilization.[38] These findings pave the way for cold plasma to be used in the following fields: wound healing, skin disorders, hospital hygiene, sterilization, anti-fungal therapies, dental care, cosmetics targeted cell, tissue elimination, and cancer treatment.[39–43] PAM has cytotoxies properties on cancerous cells while having little to no effect on normal cells.[44] In this study, we recognized CAP as a good alternative for treating cancer cells *in vitro*. The proliferation percentage of cancer cells was restricted to around 15% in 5 min plasma-treated medium.

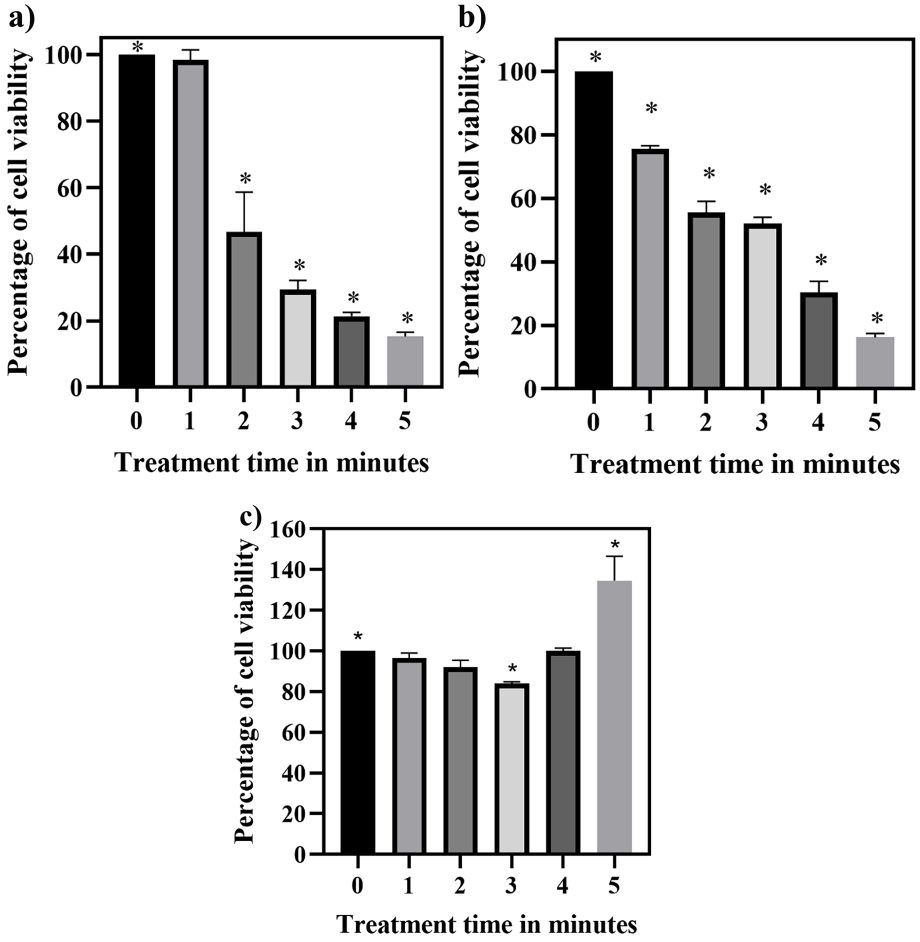

FIG. 3: (a) Percentage of viability in HeLa cells. (b) Percentage of viability in MCF-7 cells. (b) Percentage of viability in 3T3 cells. The results were normalized to 0 min. *$p < 0.05$.

In contrast, in the case of 3T3 cells, cold plasma exponentially helped in the proliferation of mouse cells. These results provide evidence of the potential of CAP to have anti-cancer properties. Similarly, the absence of 3T3 cell line inhibition by plasma treatment shows the astonishing potential of plasma-treated media to have great ability in selective inhibition of targeted cells.

C. Variability in Impacts of Plasma Treatment in Normal and Cancer Cells

The experimentally recorded data points of percent viability of cell lines (y) vs. exposure duration (x) during CAP treatment are best matched with the power function provided by Eq. (2).

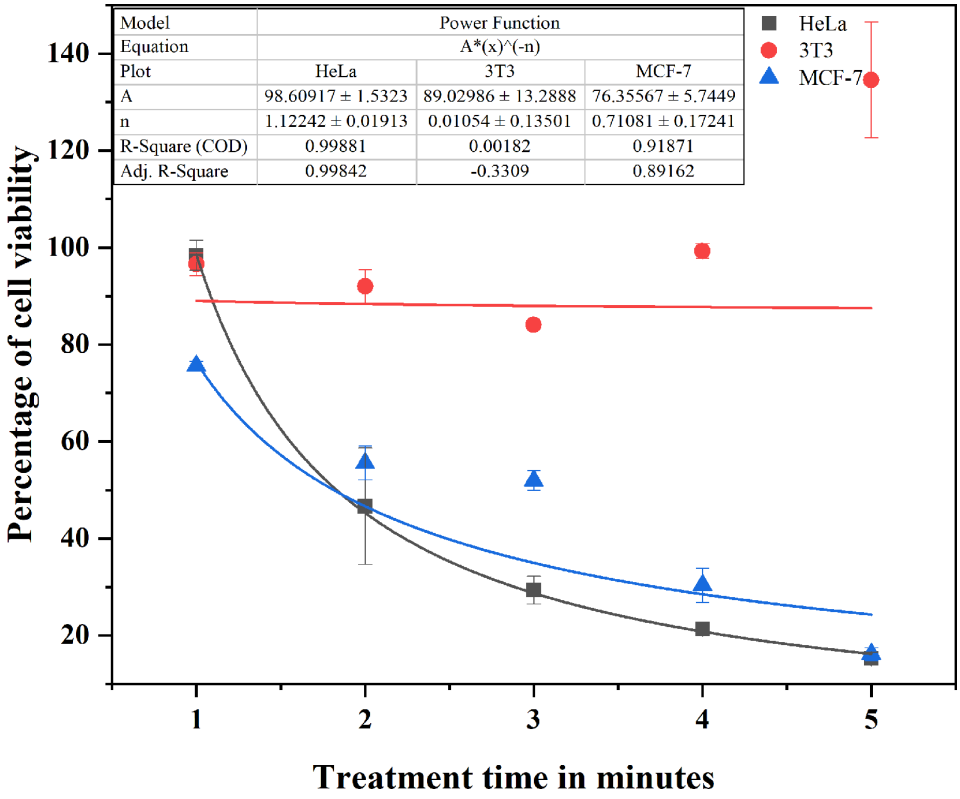

FIG. 4: Power function plot of percentage of cell viability

$$y = Ax^{-n} \quad (2)$$

where A is a constant and x is the exposure duration raised by the power (–n). A = 98.609 ± 1.532 and n = 1.122 ± 0.019 for HeLa cells. In this case, A = 89.029 ± 13.288 and n = 0.015 ± 0.135 for 3T3 cells and A = 76.355 ± 5.7, n = 0.710 ± 0.172 for MCF-7, where the included errors are the standard errors in relevant coefficients of the power functions shown in Fig. 4. The coefficient of determination, in this case, is 0.91 for MCF-7 and 0.99 for HeLa. According to the interpretation of Eq. 2 and Fig. 4, the percent viability of 3T3 cell lines declines softly with exposure time. Still, the percent viability of cancer cell lines decreases sharply. As seen in Figs. 3 and 4, PAM has significant effects on the killing of cancer cells. As a result, we may conclude that CAP selectively kills cancer cells (HeLa) rather than mouse fibroblast cells (3T3).

Along with the best fitting curve of a power function, it was discovered that the percentage of cell viability varied with the plasma treatment period. After plasma treatment, the curve for cancer cells is steeper than for mouse fibroblast cells (3T3). The quicker declining curve indicates that cancer cells were selectively killed than mouse fibroblast cells during exposure. Thus, using CAP-enabled media as a novel anti-cancer therapy is effective.[45]

We obtained cell viability for 1 min, 2 min, 3 min, 4 min, and 5 min PAM for HeLa cells to be 98.42 ± 3.040%, 46.64 ± 12.01%, 29.35 ± 2.833%, 21.35 ± 1.206%, and 15.23 ± 1.362% respectively. Similarly, for MCF-7 we obtained the cell viability as 75.62 ± 0.9207%, 55.58 ± 3.487%, 51.98 ± 2.02%, 30.37 ± 3.509%, and 16.18 ± 1.295%.

Science has discovered a unique alternative CAP that works against cancer similar to chemotherapy and radiation therapy and involves an ionization process with the production of ions, electrons, radicals, and exciting species that can eliminate cancerous cells and contribute to anti-cancer effects.[46–48] Compared to some previous methods, CAP may be administered locally, activate numerous signaling pathways in cancer cells, and have the potential for cancer cell selectivity while leaving normal cells undamaged.[7,20,49] Some of the critical findings shows that the treatment of reactive oxygen species (ROS) and reactive nitrogen species (RNS) plays a vital role in selective action against cancer cells.[48,50] The fact that CAP is very cheap to produce and portable has been one of the significant factors that the researchers will use to treat cancer.

Besides that, the mechanism behind the effectiveness of CAP against cancer is still unclear. The more research in this field to use the portability of CAP treatment compared to the traditional treatment at a low cost with high accuracy is recommended. The CAP technology using argon gas as a working environment can produce cell detachment, cause cancer cell death, and battle tumor development by improving the efficacy of antitumor treatment agents.[7,51]

D. Alterations in Radical Scavenging

DPPH is a stable free radical that is commonly used to evaluate the antioxidant activity of natural extracts or pure chemicals that function as free radical scavengers or hydrogen donors. The current investigation analyzed plasma-treated media's DPPH radical scavenging properties. We observed that the percentage of DPPH inhibition for 1 min treated PAM was 9.60 ± 11.27%, followed by 16.74 ± 16.10% and 3.342 ± 17.07% for 2 and 3 min of treatment. Whereas in 4 and 5 min, the negative results were obtained with –13.80 ± 17.85% and –25.62 ± 17.33% DPPH inhibition. The result revealed a slight increase in percentage inhibition of DPPH up to 2 min plasma-treated medium, but all other plasma-treated samples have shown a significant drop in DPPH inhibition (Fig. 5). We believe that the generation and presence of reactive species such as ROS, RNS, etc., in the treated media through the plasma discharge is one of the reasons for decreasing potential of higher-time plasma-treated media. However, various factors should be considered in affecting the activities of CAP, precisely, the types and concentrations of the components generated in CAP, which are dependent on the electrode configuration, excited voltages, generation modes, and the types of feeding gases in generating plasma.[15] There are several factors such as plasma treatment time, the different reaction gases (He, air, N_2, O_2, and gaseous mixture), the gap between liquid and plasma source, and the plasma discharge power, which should be investigated to optimize and enhance the effects of the CAP on biological activities of natural entities.[52,53]

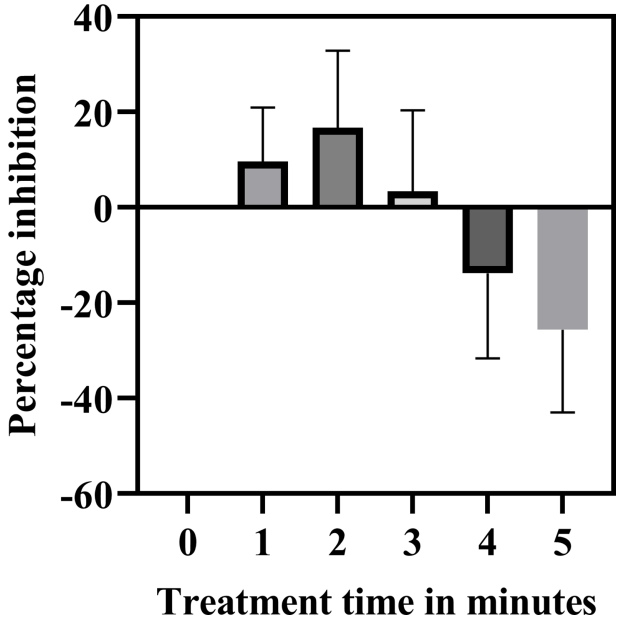

FIG. 5: Percentage of DPPH inhibition by plasma-treated media

IV. CONCLUSIONS

This study presents the development of cancer treatment based on cold plasma technology, which covers indirect CAP treatment. These CAP techniques in addition to cancer therapy, will most likely help to treat interior tissues. The optical methods are employed to characterize CAP. The electron temperature (T_e) of cold plasma has been measured to be 0.79 eV using the Boltzmann plot method. The experiments reveal that CAP has a considerable anti-cancer activity. When normal (3T3 cells) and malignant (HeLa, MCF-7) cells are treated *in vitro* with CAP, the percentage of cell viability follows the shape of a power function during the treatment period. The selectivity of plasma-treated media in inhibiting cancer cells provides CAP's great potential to have anti-cancer properties. CAP's ability in cancer treatment generates short-lived reactive species, and other characteristics such as physical components and biological effects distinguish it for further investigation. Furthermore, it is important to investigate CAP–cell interactions using microfluidics and organ-on-a chip devices.[54,55]

ACKNOWLEDGMENTS

The authors acknowledge the University Grants Commission (UGC), Sanothimi, Bhaktapur, Nepal, for providing the Master Research Support (MRS-77/78-S&T-90). The authors would also like to thank the UGC for providing a postdoctoral fellowship to S.R. (UGC Award No. PF-76/77-S&T-01). The authors declare no competing interests. Author contributions were as follows. A.S.: Designed the work, acquired laboratory

works, generated and analyzed data, and prepared the manuscript; S.P.P.: Designed the work, analyzed the data, and prepared the manuscript; N.B.: Analyzed the data and prepared the manuscript; R.S.: Designed the work and prepared the manuscript; P.R.J.: Conceived the study and the methodology for cytotoxicity studies, reviewed and edited the manuscript; A.P.: Prepared the manuscript; A.G.C.: analyzed the DPPH data; A.K.S.: provided laboratory support; R.A. and D.P.S.: supervised the project; and S.R.: conceived the study, analyzed the data, prepared the manuscript, and supervised the project.

REFERENCES

1. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57–70.
2. Cui W, Aouidate A, Wang S, Yu Q, Li Y, Yuan S. Discovering anti-cancer drugs via computational methods. Front Pharmacol. 2020;11:733.
3. Nurgali K, Jagoe RT, Abalo R. Adverse effects of cancer chemotherapy: Anything new to improve tolerance and reduce sequelae? Front Pharmacol. 2018;9:245.
4. Chen, FF. Introduction to plasma physics and controlled fusion. New York: Plenum Press; 1984.
5. Lu X, Naidis GV, Laroussi M, Reuter S, Graves DB, Ostrikov K. Reactive species in non-equilibrium atmospheric-pressure plasmas: Generation, transport, and biological effects. Phys Rep. 2016;630:1–84.
6. Li HP, Ostrikov K, Sun W. The energy tree: Non-equilibrium energy transfer in collision-dominated plasmas. Phys Rep. 2018;770–2:1–45.
7. Yan D, Keidar M, Sherman JH. Cold atmospheric plasma, a novel promising anti-cancer treatment modality. Oncotarget. 2017;8(9):15977–95.
8. Honnorat B. Application of cold plasma in oncology, multidisciplinary experiments, physical, chemical and biological modeling [Doctoral dissertation]. Paris: Sorbonne Université; 2018.
9. Goldston RJ, Rutherford PH. Introduction to plasma physics 1st ed. Florida: CRC Press; 1995.
10. Chen C, Liu DX, Liu ZC, Yang AJ, Kong MG, Chen HL, Kong MG, Kong MG, Shama G. A model of plasma-biofilm and plasma-tissue interactions at ambient pressure. Plasma Chem Plasma Process. 2014;34:403–41.
11. Kong MG, Kroesen G, Morfill G, Nosenko T, Shimizu T, Van Dijk J, Zimmermann JL. Plasma medicine: An introductory review. New J Phys. 2009;11(11):115012.
12. Keidar M. Plasma for cancer treatment. Plasma Sources Sci Technol. 2015;24:033001.
13. Samukawa S, Hori M, Rauf S, Tachibana K, Bruggeman P, Kroesen G, Whitehead, JC, Murphy AB, Gutsol AF, Starikovskaia S. The 2012 plasma roadmap. J Phys D Appl Phys. 2012;45:253001.
14. Fridman G, Friedman G, Gutsol A, Shekhter AB, Vasilets VN, Fridman A. Applied plasma medicine. Plasma Processes Polym. 2008;5:503–33.
15. Zhang R, Zhang C, Cheng X, Wang L, Wu Y, Guan Z. Kinetics of decolorization of azo dye by bipolar pulsed barrier discharge in a three-phase discharge plasma reactor. J Hazard Mater. 2007;142:105–10.
16. Shrestha R, Subedi DP, Niraula T, Pokharel M, Pandey P, Bhattarai S, Gurung JP, Shrivastava VPJG. Effect of cold atmospheric pressure argon plasma jet on wound healing. Global Sci J. 2020;8(10):1080–93.
17. Semmler ML, Bekeschus S, Schäfer M, Bernhardt T, Fischer T, Witzke K, Seebauer C, Rebl H, Grambow E, Vollmar B, Nebe JB. Molecular mechanisms of the efficacy of cold atmospheric pressure plasma (CAP) in cancer treatment. Cancers. 2020;12(2):269.
18. Shrestha R, Gurung J, Subedi D, Wong CS. Atmospheric pressure single electrode argon plasma jet for biomedical applications. Int J Emerging Technol Adv Eng. 2015;5(11):193–8.
19. Ishaq M, Bazaka K, Ostrikov K. Intracellular effects of atmospheric-pressure plasmas on melanoma cancer cells. Phys Plasmas. 2015;22(12):112–23.

20. Keidar M, Walk R, Shashurin A, Srinivasan P, Sandler A, Dasgupta S, Ravi R, Guerrero-Preston R, Trink B. Cold plasma selectivity and the possibility of a paradigm shift in cancer therapy. Br J Cancer. 2011;105(9):1295–1301.
21. Boehm D, Bourke P. Safety implications of plasma-induced effects in living cells – A review of in vitro and in vivo findings. Biol Chem. 2018;400:3–17.
22. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55–63.
23. Regmi S, Fung TS, Lim S, Luo KQ. Fluidic shear stress increases the anti-cancer effects of ROS-generating drugs in circulating tumor cells. Breast Cancer Res Treat. 2018;172:297–312.
24. Regmi S, Fu A, Luo KQ. High shear stresses under exercise condition destroy circulating tumor cells in a microfluidic system. Sci Rep. 2017;7:39975.
25. Eitan E, Zhang S, Witwer KW, Mattson MP. Extracellular vesicle–depleted fetal bovine and human sera have reduced capacity to support cell growth. J Extracell Vesicles. 2015;4(1):26373.
26. Pandey BP, Adhikari K, Pradhan SP, Shin HJ, Lee EK, Jung HJ. In-vitro antioxidant, anti-cancer, and anti-inflammatory activities of selected medicinal plants from western Nepal. Fut J Pharm Sci. 2020;6:75.
27. Wong CS, Mongkolnavin R. Elements of plasma technology. Springer Singapore. 1st ed. Gateway East: Singapore; 2016. p. 48–98.
28. Ohno N, Razzak MdA, Ukai H, Takamura S. Validity of electron temperature measurement by using Boltzmann plot method in radio frequency inductive discharge in the atmospheric pressure range. Plasma Fusion Res. 2006;1:028–028.
29. Falahat A, Ganjovi A, Taraz M, Ravari MR, Shahedi A. Optical characteristics of a RF DBD plasma jet in various Ar/O_2 mixtures. Pramana. 2018;90:1–11.
30. National Institute of Standards and Technology. Atomic spectra database version 5.9. Available from: https://physics.nist.gov/asd.
31. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, Bray F. Cancer statistics for the year 2020: An overview. Int J Cancer. 2021;149:778–9.
32. Kenari AJ, Siadati SN, Abedian Z, Sohbatzadeh F, Amiri M, Gorji KE, Babapour H, Zabihi E, Ghoreishi SM, Mehraeen RJBC. Therapeutic effect of cold atmospheric plasma and its combination with radiation as a novel approach on inhibiting cervical cancer cell growth (HeLa cells). Bioorg Chem. 2021;111;104892.
33. Terefinko D, Dzimitrowicz A, Bielawska-Pohl A, Klimczak A, Pohl P, Jamroz P. The influence of cold atmospheric pressure plasma-treated media on the cell viability, motility, and induction of apoptosis in human non-metastatic (MCF7) and metastatic (MDA-MB-231) breast cancer cell lines. Int J Mol Sci. 2021;22(8):3855.
34. Mansoori B, Mohammadi A, Davudian S, Shirjang S, Baradaran B. The different mechanisms of cancer drug resistance: A brief review. Adv Pharm Bull. 2017;7:339–48.
35. Stoffels E, Flikweert AJ, Stoffels WW, Kroesen G. Plasma needle: A non-destructive atmospheric plasma source for fine surface treatment of (bio)materials. Plasma Sources Sci Technol. 2002;11:383–8.
36. Laroussi M, Hynes W, Akan T, Lu X, Tendero C. The plasma pencil: A source of hypersonic cold plasma bullets for biomedical applications. IEEE Trans Plasma Sci. 2008;36:1298–9.
37. Lu X, Zhonghe J, Xiong Q, Tang Z, Pan Y. A single electrode room-temperature plasma jet device for biomedical applications. Appl Phys Lett. 2008;92:151504.
38. Lu X, Jiang Z, Xiong Q, Tang Z, Hu X, Pan Y. An 11 cm long atmospheric pressure cold plasma plume for applications of plasma medicine. Appl Phys Lett. 2008;92:081502.
39. Fridman G, Shereshevsky A, Peddinghaus M, Gutsol A, Vasilets V, Brooks A, Balasubramanian M, Friedman G, Fridman A. Bio-medical applications of non-thermal atmospheric pressure plasma. 37th AIAA Plasmadynamics and Lasers Conference. 2006 Jun 5–8; San Francisco, California; 2006. p. 2095–8.
40. Morfill GE, Kong MG, Zimmermann JL. Focus on plasma medicine. New J Phys. 2009;11:115011.

41. Zirnheld JL, Zucker SN, DiSanto TM, Berezney R, Etemadi K. Nonthermal plasma needle: Development and targeting of melanoma cells. IEEE Trans Plasma Sci. 2010;38(8):948–52.
42. Georgescu N, Lupu AR. Tumoral and normal cells treatment with high-voltage pulsed cold atmospheric plasma jets. IEEE Trans Plasma Sci. 2010;38:1949–55.
43. Stofels E, Sakiyama Y, Graves DB. Cold atmospheric plasma: Charged species and their interactions with cells and tissues. IEEE Trans Plasma Sci. 2008;36:1441–57.
44. Zahedian S, Hekmat A, Tackallou SH, Ghoranneviss M. The impacts of prepared plasma-activated medium (PAM) combined with doxorubicin on the viability of MCF-7 breast cancer cells: A new cancer treatment strategy. Rep Biochem Mol Biol. 2022;10(4):640–52.
45. Soni V, Adhikari M, Simonyan H, Lin L, Sherman JH., Young CN, Keidar MJC. In vitro and in vivo enhancement of temozolomide effect in human glioblastoma by non-invasive application of cold atmospheric plasma. Cancers. 2021;13(17):4485.
46. Jonkman JEN, Cathcart JA, Xu F, Bartolini ME, Amon JE, Stevens KM, Colarusso P. An introduction to the wound healing assay using live-cell microscopy. Cell Adhes Migr. 2014;8:440–51.
47. Babaeva NY, Naidis GV. Modeling of plasmas for biomedicine. Trends Biotechnol. 2018;36:603–14.
48. Von Woedtke T, Schmidt A, Bekeschus S, Wende K, Weltmann KD. Plasma medicine: A field of applied redox biology. In Vivo. 2019;33:1011–26.
49. Siu A, Volotskova O, Cheng X, Khalsa SS, Bian K, Murad F, Keidar M, Sherman JH. Differential effects of cold atmospheric plasma in the treatment of malignant glioma. PLoS One. 2015;10:0126313.
50. Babington P, Siu A, Sherman JH, Rajjoub K, Canady J, Keidar M. Use of cold atmospheric plasma in the treatment of cancer. Biointerphases. 2015;10(2):029403.
51. Brany D, Dvorska D, Halasova E, Skovierova H. Cold atmospheric plasma: A powerful tool for modern medicine. Int J Mol Sci. 2020;21:2932.
52. Cheng X, Murphy W, Recek N, Yan D, Cvelbar U, Vesel A, Mozetič M, Canady J, Keidar M, Sherman JH. Synergistic effect of gold nanoparticles and cold plasma on glioblastoma cancer therapy. J Phys D Appl Phys. 2014;47(33):335402.
53. Shrestha R, Subedi DP, Adhikari S, Maharjan A, Shrestha H, Pandey GRJM. Experimental study of atmospheric pressure argon plasma jet−induced strand breakage in large DNA molecules. Plasma Med. 2017;7(1):65–76.
54. Regmi S, Fu A, Lim S, Luo KQ. Destruction of circulating tumor cells by fluid shear stresses generated in a microfluidic system. Cancer Res. 2017;77(13):2325.
55. Regmi S, Poudel C, Adhikari R, Luo KQ. Applications of microfluidics and organ-on-a-chip in cancer research. Biosensors. 2022;12(7):459.