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Microplasma Effect on Skin Scaffold for Melanoma Cancer Treatment

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Abstract. An atmospheric plasma system using Helium gas was developed. The effect of helium plasma treatment on skin scaffold surface was studied by scanning electron microscopy (SEM). The changes of skin scaffold surfaces before and after helium plasma treatment was recorded. The surface of skin scaffold changed with the prolonged of helium plasma treatment time. The depth of helium plasma penetration was studied using methylene blue dye staining method. The methylene blue will detect the presence or absence of an oxygen that was induced from plasma excitation. The presence of the oxygen indicated on the depth of helium plasma penetration. Results showed plasma are able to penetrate 4mm of skin scaffold after 1200 seconds of exposure.

INTRODUCTION

Plasma is an ionized gas, and is distinct as fourth state of matter. “Ionized” mean that at least one electron is not certain to an atom or molecule, converting the atoms or molecules into positively charged ions. As temperature increases, molecules become energetic and transform matter in the sequence: solid, liquid, gas and finally plasma [1], [3].

Plasma occur naturally but also can be effectively man-made in laboratory and in industry, which provides opportunities for numerous application, including thermonuclear synthesis, electronic laser, fluorescent lamps, and many more [1], [3].

A plasma source in most laboratory environment is a gas discharge. A simple electric discharge can be described as two electrodes inserted into a glass tube and connected to a power supply. As the voltage applied across the two electrodes increases, the current suddenly increase sharply at a certain voltage required for sufficiently intensive electron falls [1], [3], [11]. The reactive species ignited by the plasma excitation will make the plasma treatment capable to be used in large application fields especially in cancer treatment.

The rate of melanoma skin malignancy has expanded in numerous part of the world and it is one of the primary driver of death from tumor [5], [6], [7]. Prior to this, treatment of melanoma depended on surgical or chemotherapy specialists but it is frequently incurable [7]. Atmospheric pressure plasma may offer alternative treatment for cancer therapy [4].

One of the suggested method to treat human melanoma skin cancer is by applying the atmospheric plasma treatment. In the past, the effect of plasma on cell motility and expression of cell migration was investigated and it shows the ability of plasma treatment in reducing the cancer cell viability and inducing the apoptosis [9], [15], [20]. The aim of this research is to study on the effect of microplasma to skin scaffold in terms of depth penetration.

Human skin consists of two layers, upper epidermis and underneath dermis layer. There are a few methods for developing the model of the human skin layer such as by scaffolding, cell culture and cell harvesting. Scaffolding is

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the best method compared to cell culture and cell harvesting to develop an artificial skin. Melanoma is a malignant tumor of melanocytes, which are the cells that make the pigment melanin and are derived from the neural crest. Most melanomas arise in the skin [7]. The risk profile and extent of melanoma spread is described as staging [2], [7]. At the later stage as shown in Figure 1, the melanoma grows deeper into the skin.

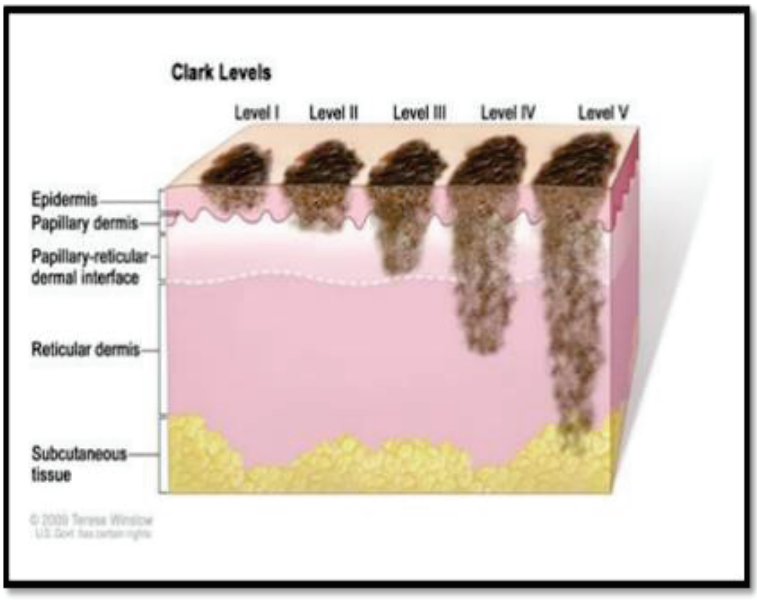


FIGURE 1: Human Melanoma Skin Cancer. Adapted with permission from National Cancer Institute by Terese Winslow. Copyright (2009)

G.Fridman et al showed that plasma can kill melanoma skin cancer cells through necrosis at higher treatment doses (15s of treatment time). Plasma treatment initiates apoptotic behavior in cells not through poisoning of the growth media in which the cells reside but through direct interaction with the cells [3]. In addition, Anke Schimidt et al also observed that plasma is composed of multiple chemically active species that can be cytotoxic or cause cell death [9]. This active species can cause changes in cellular morphology and mobility. When plasma applied on melanoma cells, apoptosis was induced in cancer cells due to the active species. Prior to this, it can be showed that plasma treatment are able to be used for cancer therapy.

METHODOLOGY

Figure 2 shows the flowchart for this research. It starts with plasma generation set up, experiment with skin scaffold and also analysis of the surface of the scaffold.

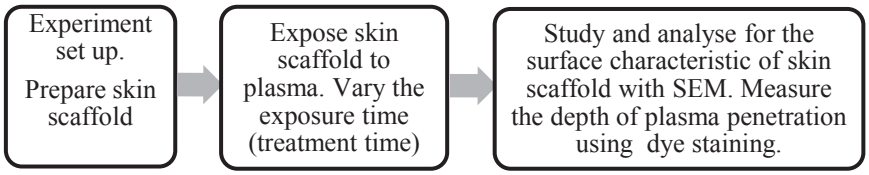


FIGURE 2: Experiment flowchart

Experiment Setup

The power supply is a device that supplies the electric power to the electric load. It converts the ac or dc power into the required voltage. A pulse inverter that converts 24VDC 10A to 10KV AC was utilized. A flow meter is used to control the gas flow through the glass tube. It used helium gas as working gas and it flows at constant flow rate

controlled by a lab view software. The helium gas was selected as working gas because it is most convenient gas while working at atmosphere pressure. It allows stabilizing homogeneous glow discharges much easier compared to other gases at lower voltages [14]. Figure 3 shows the plasma system setup.

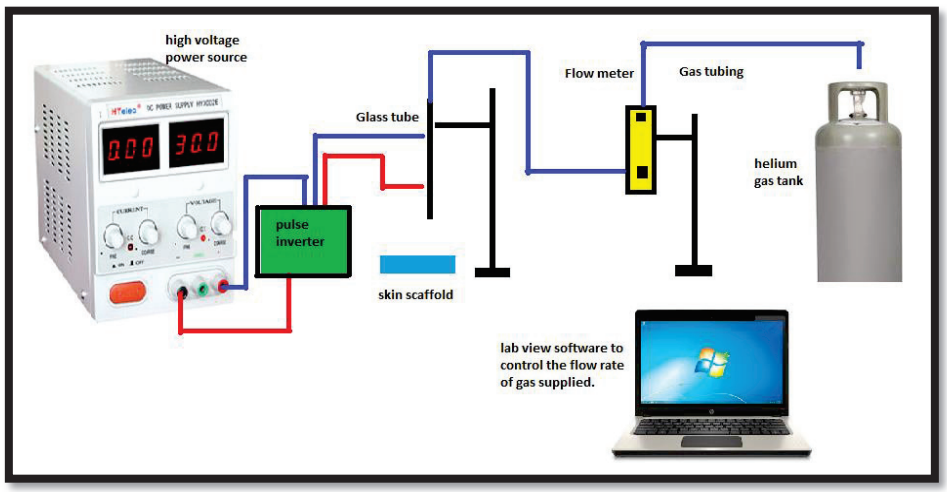


FIGURE 3*:* Plasma set up

Skin Scaffold Preparation

Chitosan, sodium chloride and gelatine were all combined to build a scaffold that mimic both dermis and epidermis to represent the skin layers. A solution of chitosan, sodium chloride and gelatine were variegated with final concentrations of 93.75 µM, 0.05 µM and 2 mM, separately [17].

The final concentration of solutions (M) need to prepare by calculating the exact mass of the solute used. By applying the molarity formula in equation (1), we determine the moles of solute. Then, we calculate the molar mass for each material based on its molecular formula. The molar mass of components are calculated by taking an atomic mass of the component from the periodic table. Lastly, by applying the moles of solute in equation (2), we determine the mass of solute (g).

$$\text{Molarity (M)} = \frac{\text{Moles of salute (mole)}}{\text{Litre of solution (L)}} \tag{1}$$

$$\text{Moles of solute (mole)} = \frac{\text{Mass of solute (g)}}{\text{Molar mass of solute } (\frac{\text{g}}{\text{mole}})} \tag{2}$$

After mixing the solutions of chitosan, sodium chloride and gelatine, the variegated solution was transferred gently into a petri dish and frozen at -20◦C for 24 h. The scaffold size could be controlled by adjusting the working volume of the formulating solution. The chitosan/sodium chloride/gelatine sponge was assembled by lyophilizing the frozen solution for 24 h. It was then chemically cross-linked for 24 h at 25◦C in pure ethanol having 50 mM Glutaraldehyde (GH). Afterwards, the reaction was completed by taking out the GH solution and washed with distilled water for more three times to eliminate un-reacted chemicals. The scaffold was lyophilized for another 24 h [17]. The scaffold developed with 4mm of thickness as shown in figure 4. This is similar to the thickness of the real human skin layer from epidermis to subcutaneous tissue.

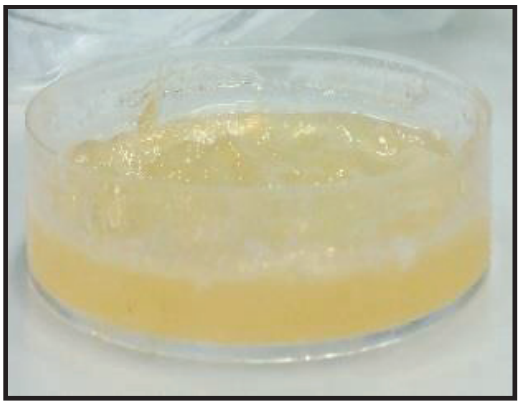


FIGURE 4: Skin scaffold development

Plasma Treatment on Skin Scaffold

After the plasma system setup is completed, a skin scaffold is exposed for plasma treatment. The scaffold were exposed to plasma from 60 seconds to 150 seconds with the supply of 350 milliliter/minute of helium gas. The spacing between two electrodes (high voltage and ground) is 2 cm while the space between glass tube and sample is 1 cm as shown in figure 5.

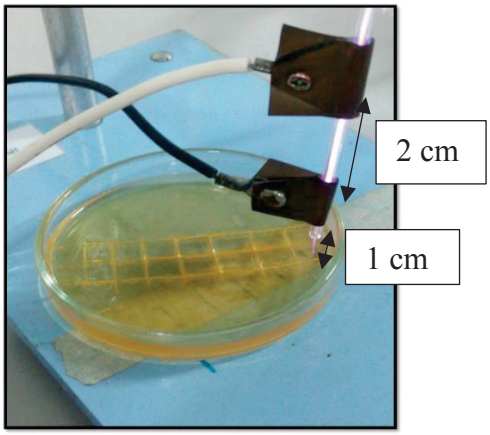


FIGURE 5*:* Spacing between electrode and float glass in experiment

After the skin scaffold is exposed to plasma, the scaffold is taken for SEM (JEOL, JSM, and 6460 LA) viewing. The distance between lenses and specimen is 4.4 with accelerating voltage of 15000 Volt. The working distance is 10 µm with vacuum condition of 15.0 kV.

Measuring the Depth of Plasma Penetration

Plasma will induce the reactive species such as oxygen. Detection of this reactive oxygen species indicates how far the penetration of plasma into skin scaffold. A dye staining is used to detect the presence of reactive species. In this experiment, a dye stain, methylene blue was used to detect the presence of oxygen [10]. One drop of methylene blue was placed on the skin scaffold before exposing to the plasma as shown in figure 6. The absorption of methylene blue by a skin scaffold indicated that there are presence of an oxygen species [10]. The absorption methylene blue was observed and measured to study the depth of plasma penetration.

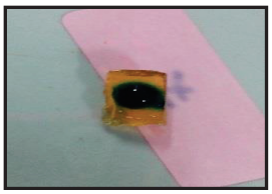
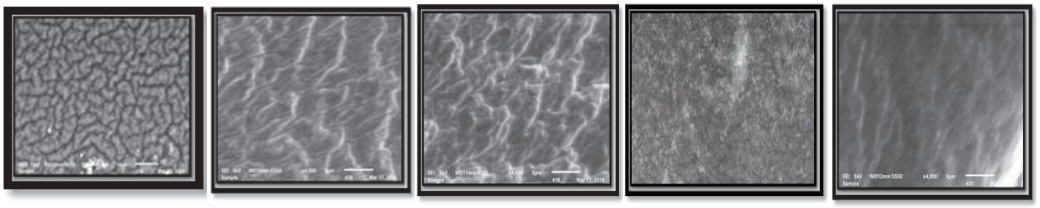


FIGURE 6*:* Methylene Blue on Skin Scaffold*.*

RESULTS AND DISCUSSION

Result of Scanning Electron Microscope (SEM)

The texture on the cleaned skin scaffold surfaces is observed and viewed under SEM with magnification X1700. From the Figure 7, it is clear that the surfaces of skin scaffold before treatment contain some pores and the texture of the scaffold surface has changed after treatment. The texture of the skin scaffold surface become smoother when the treatment time was longer. The figure clearly shows the difference texture of the skin scaffold with shorter time, 60 seconds and longer time, 150 seconds. 150 seconds of plasma exposure appears to have a smoother surface compared to 60 seconds.

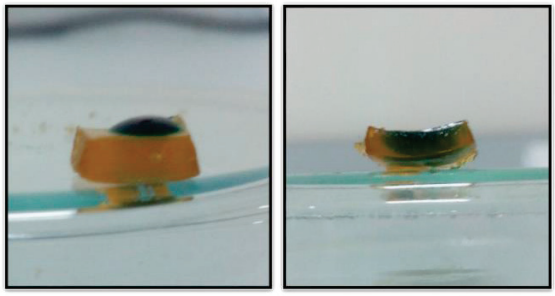


Before treatment 60 sec 90 sec 120 sec 150 sec

FIGURE 7*:* Surface of skin scaffold from SEM images.

Observation of Depth Plasma Penetration

The depth of plasma penetration were observed using the measurement of methylene blue absorption through the skin scaffold. Figure 8 shows example of the methylene blue absorption and Table 1 shows the results depth penetration of methylene blue.



Before treatment After treatment (1200s of microplasma exposure)

FIGURE 8: Images of methylene blue absorption

Table 1. The depth penetration measurement of methylene

Treatment time (sec)	Depth of plasma penetration (mm)
60	No penetration
90	0.1
120	0.2
150	0.4
300	1
600	2
900	3
1200	4

Helium plasma produced the excited species and ions which was directed onto the skin scaffold. When the excited species is deposited to air, the air molecules become excited [8], [12]. As air is composed primarily of nitrogen and oxygen, excited N_2 and O_2 molecules are produced. These can react with other molecules, forming mainly ozone and nitrogen (II) oxide. The reactive species present in the plasma can readily react with other chemicals present in the air or on nearby surfaces. The presence of oxygen molecule on the surfaces used to study the depth of helium plasma penetration. A dye staining, methylene blue was used to detect the presence of oxygen molecules [10]. During plasma exposure, the skin scaffold will absorb the methylene blue and this absorption will indicate the presence of oxygen molecule. The length of methylene blue absorption indicated the difference depth of plasma penetration.

From the table 1, it is clear that the level of methylene blue absorption through skin scaffold differed between 60 seconds to 1200 seconds of helium plasma time exposure. It shows that helium plasma can penetrate far through skin scaffold if the time for helium exposure is long. From previous studies, it said that 60 seconds of time plasma exposure enough for apoptosis of skin cancer cell [3], [9], [21]. Our findings shows that treatment time that is less than 60s did not penetrate trough the skins scaffold. It can be concluded that in our plasma setup, longer treatment time will be needed to penetrate into skin scaffold.

CONCLUSION.

The scaffold were divided to two different tests which are for surface morphology analysis and the depth of penetration observed. Observation on the surface morphology was done by using the SEM after the scaffold was treated to plasma with different of the treatment time. The observation from the SEM image shows how the treatment time affect the changes of the surface. The depth of plasma penetration was observed using methylene blue. The methylene blue detected the presence of an oxygen that induced from plasma excitation. Detection of oxygen indicated on how far the plasma can penetrate through a skin scaffold. The penetration can go as deep as 4mm of the skin scaffold thickness. A melanoma is 4mm deep. This shows the potential of plasma to treat the melanoma skin cancer.

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REFERENCES

[1] Mohd., N.D., Abu Bakar, S., Zaaba, S.K., Wan Ahmad, W.K., "Study on bovine bone surface after atmospheric plasma treatment", Proceedings of Biomedical Engineering and Sciences 2012 (IECBES), 17-19 Dec 2012.

[2] Siegel, R., Ma, J., Zou, Z. and Jemal, A., "Cancer statistics, 2014", CA: A Cancer Journal for Clinicians, 64: 9–29, (2014)

[3] Fridman, G., Shereshevsky, A., Jost, M. M., Brooks, A. D., Fridman, A., Gutsol, A., Friedman, G. Floating electrode dielectric barrier discharge plasma in air promoting apoptotic behavior in melanoma skin cancer cell lines. Plasma Chemistry and Plasma Processing, 27(2), 163-176, (2007).

[4] Keidar, M., Shashurin, A., Volotskova, O., Ann Stepp, M., Srinivasan, P., Sandler, A., & Trink, B. (2013). Cold atmospheric plasma in cancer therapy. Physics of Plasmas, 20(5). http://doi.org/10.1063/1.4801516

[5] Rebecca Siegel, Deepa Naishadham and Ahmedin Jemal, "Cancer statistics, 2013", CA: A CANCER JOURNAL FOR CLINICIANS, 63 (1): 11–30, (2013)

[6] Rebecca Siegel, Deepa Naishadham and Ahmedin Jemal, "Cancer statistics, 2012", CA: A CANCER JOURNAL FOR CLINICIANS, 62 (1): 10–29, (2012)

[7] American Cancer Society: Cancer Facts and Figures 2016. Atlanta, Ga: American Cancer Society, 2016. Available onlineExit Disclaimer. Last accessed December 8, 2016.

[8] J. Y. J. Andreas Sch¨utze, Steven E. Babayan, JaeyoungPark, Gary S. Selwyn, and Robert F. Hicks, (1998). The Atmospheric-Pressure Plasma Jet: A Review and Comparison to Other Plasma Sources, IEEE Transaction on Plasma Science, vol. 26, pp. 1685-1694.

[9] Anke Schmidt, Sander Bekeschus, Thomas Von Woedtke, Sybille Hasse, “Cell Migration and Adhesion of a Human Melanoma Cell Line”.2015:21.

[10] Dikalov, S., & Harrison, D. G. (2014). Methods for detection of mitochondrial and cellular reactive oxygen species. Antioxidants & Redox Signaling, 20, 372–382.

[11] Ionita, E. R., Ionita, M. D., Stancu, E. C., Teodorescu, M., & Dinescu, G. (2009).Small Size Plasma Tools for Material Processing at Atmospheric Pressure, Applied Surface Science, 255(10), 5448-5452.
[12] Nie, Q. Y., Yang, A., Wang, Z. B., Li, H. P., Zhang, X. Z., & Bao, C. Y. (2012). Characteristics of Atmospheric Room-Temperature Argon Plasma Streams Produced using A Dielectric Barrier Discharge Generator with A Cylindrical Screwlike Inner Electrode.
[13] Stancu, E., Stanciuc, A., Ionita, M., Teodorescu, M., Moldovan, L., & Dinescu, G. Atmospheric Pressure Plasma Preparation of Micropatterned Teflon Surfaces For Interaction with Cells.
[14] Li, X., Tao, X., & Yin, Y. (2009). An Atmospheric-Pressure Glow-Discharge Plasma Jet and Its Application. Plasma Science, Ieee Transactions on, 37(6), 759-763.
[15] Shintani, H., Sakudo, A., Burke, P., & Mcdonnell, G. E. R. A. L. D. (2010). Gas Plasma Sterilization of Microorganisms And Mechanisms of Action.Experimental And Therapeutic Medicine, 1(5), 731.
[16] Park, B. J., Seo, H. J., Kim, J., Kim, H. L., Kim, J. K., Choi J. B. & Park, J. C. (2010). Cellular Responses Of Vascular Endothelial Cells on Surface Modified Polyurethane Films Grafted Electrospun PLGA Fiber With Microwave-Induced Plasma At Atmospheric Pressure. Surface and Coatings Technology, 205, S222-S226.
[17] Liu, H., Mao, J., & Yao, K. (2012). Journal of Biomaterials Science , Polymer Edition A study on a chitosan-gelatin- hyaluronic acid scaffold as artificial skin in vitro and its tissue engineering applications, (January 2013), 37–41.
[18] Borcia, C., Dumitrascu, N., & Borcia, G. (2012). Comparing The Modification Induced By Plasma and Uv Radiation To Polymer Surfaces. Romanian Reports in Physics, 64(1), 163-172.
[19] France, R. M., & Short, R. D. (1994). Plasma Treatment of Polymers: Effects of Energy Transfer from an Argon Plasma and Post-Plasma Storage on the Surface Chemistry of Polystyrene. Polymer Degradation and Stability, 45(3), 339-346.
[20] Baier, M., Janßen, T., Wieler, L. H., Ehlbeck, J., Knorr, D., & Schlüter, O. (2015). Inactivation of Shiga toxin-producing Escherichia coli O104: H4 using cold atmospheric pressure plasma. Journal of Bioscience and Bioengineering, xx(xx), 5–9. http://doi.org/10.1016/j.jbiosc.2015.01.003
[21] Babington, P., Rajjoub, K., Canady, J., Siu, A., Keidar, M., Sherman, J. H.Siu, A. (2015). Use of cold atmospheric plasma in the treatment of cancer Use of cold atmospheric plasma in the treatment of cancer, 29403. http://doi.org/10.1116/1.4915264