Jurnal Teknologi

Full Paper

AN OVERVIEW: INVESTIGATION OF ELECTROPORATION TECHNIQUE ON CELL PROPERTIES CULTURED ON MICROPATTERNED SURFACE.

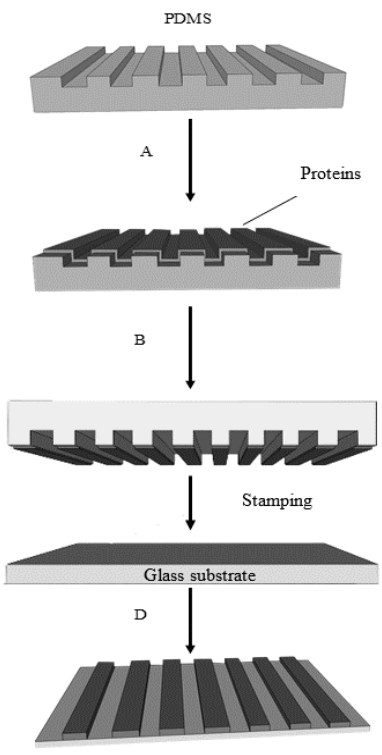
Nur Adilah Abd Rahman, Mamman Hassan Buhari, M. Mahadi Abdul Jamil*.

Biomedical Modelling and Simulation Research Group, Faculty of Electrical and Electronics Engineering, Universiti Tun Hussein Onn Malaysia, Batu Pahat, Johor, Malaysia.

Article history
Received
19 June 2015
Received in revised form
26 June 2015
Accepted
10 July 2015

*Corresponding author
drmuhammadmahadi@gmail.com

Graphical abstract



Abstract

Electroporation (EP) is a method of controlling cell function by using pulses of electrical fields to create pore through a cell membrane and causes other substance around it to be absorbed into the cell. Where This method had been led to variety of medical applications. While, microcontact printing (μCP) is a quite useful technique for patterning extracellular matrix as an adhesion molecule for cells that works for controlling the cell growth. This study focuses on reviewing the basic concepts and techniques of electroporation and Microcontact printing, as applied to molecular biology & cancer treatment. The combination of these two technique might be a new technique for wound healing process treatment.

Keywords: Electroporation, microcontact printing, cell proliferation, wound healing.

Abstrak

Electroporation (EP) adalah satu kaedah mengawal fungsi sel dengan menggunakan denyutan medan elektrik untuk mewujudkan liang pada sel membran dan menyebabkan bahan lain di sekelilingnya untuk diserap ke dalam sel. Di mana kaedah ini telah membawa kepada pelbagai aplikasi perubatan. Manakala Percetakan Microcontact (μCP) adalah satu teknik yang agak berguna untuk mencorak extracellular matriks sebagai molekul lekatan untuk sel-sel yang berfungsi untuk mengawal pertumbuhan sel. Kajian ini tertumpu kepada kajian konsep-konsep asas dan teknik electroporation dan juga percetakan Microcontact, sebagaimana yang telah di guna pakai untuk biologi molekul & rawatan kanser. Dengan gabungan kedua-dua teknik ini mungkin dapat menghasilkan satu teknik baru untuk rawatan luka dalam proses penyembuhan.

Kata kunci: Electroporation, percetakan microcontact, percambahan sel, penyembuhan luka.

1.0 INTRODUCTION

This review describes techniques of Electroporation and Microcontact printing on several application and some study on research literature review. Where this two method are widely used on biomedical engineering applications. The biological effects of microsecond pulse electric fields (μsPEF) had been intensively investigated over the last few decade [1]. Electroporation is a viable physical technique, where it is in high intensity, short duration pulses are applied to temporarily open up pores in the plasma membrane of the cells to allow the transport of therapeutic materials including drugs, antibodies and genes (DNA) which otherwise are impermeable [2]. While, C.chen et al. had been studying the biological effect of μsPEF and found that the increasing of transient in the permeability of the cell membrane is used to introduced DNA or other molecules into the cells. This phenomenon is potentially, the basis for many applications in vivo such as electrochemotherapy and gene therapy, ex vivo involve blood cells, treated outside of the body with reintroduction to body to provide therapy, and also in vitro used to transfect suspended or anchored cells in laboratory apparatus by introduced DNA [3]. By applying the electrical pulses across the cells, a variety of outcomes that is from no effects to reversible EP and to irreversible EP depending on the range of electrical pulses applied to it [4].The method of EP will be combined with the μCP as the technique of micropatterned surface will be used the Microcontact printing method. Microcontact printing is a remarkable surface patterning technique. That was developed about 10 years ago and has triggered enormous interest from the surface science community, as well as from engineers and biologist [5]. It was quite a useful technique for patterning extra-cellular matrix (ECM) that works as an adhesion molecule for cells [6]. Research on EP and μCP has been important in the improvement of biomedical application and future technologist in tissue engineering fields and wound healing research. There is still no research on combination of these two method that had been done so far.

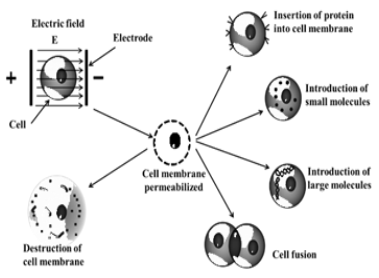


Figure 1 Different application of single cell [46].

2.0 LITERATURE REVIEW

2.1 Electroporation

Somphop Rodamporn, 2011 state that electroporation is a widely used method for introducing foreign materials (gene, DNA, RNA etc.) into tissue, animal cells and plant cells [1-3]. The main object of electroporation is to employ electric field for opening pores and facilitating gene and DNA into cell or tissue. Since Eberhard Neumann used an electroporation system in molecular biology, many of electroporation devices have been developed [2]. In comparison to other method of gene transfer, such as non-viral vector and viral vector, electroporation is a noninvasive and nonchemical method. It has many advantages for example, this method does not change the biological structure and function of the target cells, and it is safer, high efficiency and less immunologic [6]. R. Sundararajan. et. al. 2011 had made a research on the effect of irreversible electroporation on cancer cells. Where he state that the usage of electrical pulses along with drugs – electrochemotherapy (ECT) is a fast rising option for the treatment of chemo-refractive cancers. The applied electrical pulsed can be of two types either for reversible and irreversible electroporation of the cells. Reversible electroporation is primarily used for delivery of molecules into the cell. While, irreversible electroporation could be applied as in the absence of chemo drug to kill the cancerous cells. Gouping Guan et. al. also had mention that there are few reports focusing on the decorations of in vivo environment to implanted biomaterials and the effects of the decoration on cells proliferation and wound healing [7].

2.2 Microcontact Printing

The recent development of biological applications of μCP (from cell biology, tissue engineering, cell cocultures, bio-assays, bio sensors, etc.) it led to huge burst of technical papers in the last 10 years, by providing adaptation of micropatterning techniques to various substrates (glass, plastics, hydrogels, elastomers, etc.), molecules, and cell types, in two dimensions (2D) and three dimensions (3D) [8]. It has triggered enormous of interest from the surface science community, as well as from engineers and biologists. The technique of patterning for used to control both the adsorption of proteins to these surfaces and the attachment of cells to them. The ability to generate patterns of proteins and cells on surface is important for biosensor technology, tissue engineering, and fundamental studies of cell biology. The placement of biological ligands at well-defined locations on substrates is required for certain biological assays, for combinatorial screening, and for the fabrication of biosensors. Control over the positioning of cells is also important for cell-based

screening, in which individual cells need to be accessed repeatedly to perturb them and to monitor their response. Tissue engineering may require that cells to be placed in specific locations to create organized structures. The patterning techniques is controlled both the size and shape of the cell anchored to a surface, and chemistry and topology of the substrate to which the cell is attached, are also extremely useful in understanding the influence of the cell-material interface on the behavior of cells [9].

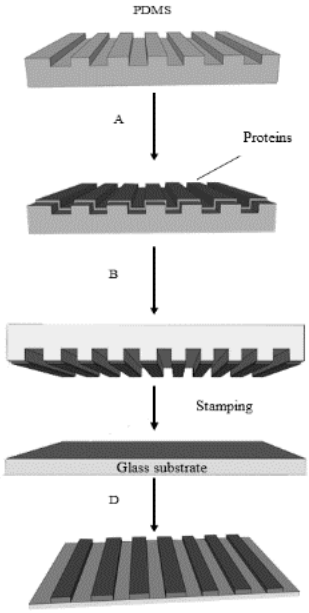


Figure 2 Stamping method

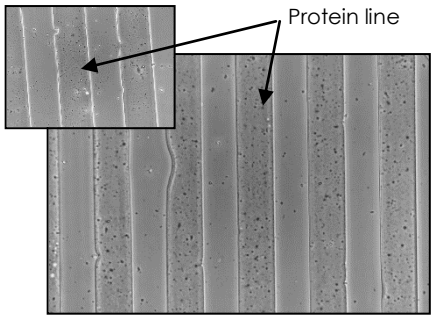


Figure 3 Result from the stamping method (50µm)

3.0 TECHNIQUES

Electroporation is the technique that utilizes of applying high magnitude electric pulses (thousands of V/cm) to induce permeability increase in cell membrane [10], short duration pulses to open up pores, allowing the passage of chemo drugs that are normally impermeable or less permeable through the cell plasma membranes [11]. EP generally depends upon the intensity of the electric field, the duration of each pulse, the number of pulses and the interval between them. Depending upon the magnitudes of these parameters, there are three effects on the cell membrane. Either, no changes on the cell membrane, temporary opening of the cell membrane after which the cell can still survive (reversible EP), or permanently open the cell membrane and the cell dies after irreversible EP (IRE). The upper limits to the range of electrical parameters that induced reversible EP is IRE. Thus, IRE causes permanent permeabilization of the cell membrane and the consequent loss of the cell homeostasis, when an electrical field is applied to cancer cells. The advantages of IRE are that no drugs are used and it is non thermal mechanism of action, dependent on the blood flow, allows focal tissue ablation and requires a short application time [11]. While, µCP is the technique that has been used most extensively for patterning proteins and cells. For example, photolithography can be used to generate pattern by photo ablating proteins preadsorbed to a silicon or glass surface [12]. This technique of microcontact printing are not expensive, it was simple procedure, it can be used to pattern variety of different planar and non-planar substrates, and this technique also do not require stringent control over the laboratory environment for their successful application.

4.0 APPLICATIONS

EP and µCP have been in application in different areas in biotechnology, medicine and biologist, where EP has been used clinically for more than 15 years in combination with chemotherapy, and is on the rise as a monotherapy over the last five years [10]. The applications of EP is included gene therapy, chemotherapy, cell fusion (used in immunology and cloning research) and sterilization of beverages and water [13]. The introduction of electroporation to cancer therapy originated from the drug delivery applications. Specifically, electroporation was used in order to assist uptake of chemotherapeutic drug molecules into the tumor cells [14]. By the pairing of electroporation and chemotherapeutic drug quickly gained popularity and now exists as an independent treatment termed electrochemotherapy (ECT) which is already in clinical practice [10]. While, µCP has proven to be a useful technique in the patterned functionalization of certain chemicals onto surfaces. It has been particularly valuable in the patterning of biological materials [15]. Where is, µCP application are wide ranging including microelectronics, surface chemistry and cell biology. It significantly had a large impact on the study and control cell growth [16]. It also has been used to directly pattern arrays of proteins on silicon or glass substrates. Furthermore, µCP can be used to directly print bacterial or mammalian cells [17]. One of the most potent applications of µCP is the fabrication of microchips for use in bio- or chemical sensors, a catalytic

surfaces, polymers and biomolecules, nano-elecronics, μCP-patterned SAMs used as resist and templates and cell biology [18].

5.0 CONCLUSION

The basic concepts and techniques of Electroporation and Microcontact Printing were highlighted. Both EP and μCP were found to be related to wound healing processes, depending on the level of their threshold and application (gene therapy, electrochemotherapy, and wound healing or tissue ablation). Investigations on EP and μCP showed that the two methods can be combined in in-vivo studies to see the cell response to μCP with the PEF applied on it. Some factors are need to be considered while selecting EP device and μCP method/technique, which include the cell type, volume, transfer molecule type and system configuration. The result of this research may lead to the development of wound healing and skin cancer treatment.

Acknowledgement

The author wish to acknowledge the UTHM GIPS grant sponsor. We would also like to thank to Supervisor Associate professor Dr. Muhammad Mahadi for the guidance.

References

[1] Mohamad Nazib Adon, M. Noh Dalimin, Muhammad Mahadi Abdul Jamil, Norazan Mohd Kassim, Sallehhuddin Hamdan. 2012. Study of Effect of Microsecond Pulsed Electric Fields on Threshold Area of HeLa cell. *IEEE EMBS International Conference on Biomedical Engineering and Sciences*, Langkawi, 17th- 19th December 2012.

[2] R. Sundrarajan, T. Salameh, I. Camarillo and L. Campana. 2011. Effective Use of Electrical Pulses on Cancer Cells to Control Proliferation. *IEEE*.

[3] James C. Weaver. 2000. Electroporation of Cells and Tissues. *Invited Paper, IEEE Transactions on Plasma Science*. 28(1): February.

[4] Antoni Ivorra and Boris Rubinsky. 2006. Impedance Analyzer for in vivo electroporation studies. *Proceedings of the 28th IEEE, EMBS Annual International Conference*, New York City. Aug 30 – Sept 3. 5056 – 5059.

[5] S. Wu, J. Guo, B. Su, J. Zhang, J. Fang. 2013. Nanosecond Pulsed Electric Fields Adjuvant Chemotherapy for Breast Cancer: An In Vivo Study. *IEEE 2013*.

[6] Somphop Rodamporn. 2011. Optimal Parameters of Electroporation for Gene and Tissue. *The 2011 Biomedical Engineering International Conference (BMEiCON-2011). IEEE*. 279 -282.

[7] R. Sundararajan, Ramyan Rajendran, Sajan S. Shahid, Santosh D.K, Snehalatha Radhakrishnan, Priyadarshan K., Varsha S., U. Vimal Kumar, Rajaprabu Ramachandran, and Kavita Sankaranarayanan. 2011. Effect Of Irreversible Electroporation On Cancer Cells. *Electrical Insulation and Dielectric Phenomena (CEIDP), 2011 Annual Report Conference. IEEE*. 16-19 Oct. 2011. 164 – 167.

[8] Ammar Azioune, Nicolas Carpi, Qingzong Tseng, Manuel Thery, and Matthieu Piel. 2010. Protein Micropatterns: A Direct Printing Protocol Using Deep Uvs. Chapter 8, *Method in Cell Biology*. 97.

[9] Ravi s. Kane, Shuichi Takayama, Emanuele Ostuni, Donald E. Ingber, George M. Whitesides. 1999. Patterning Proteins and Cells Using Soft Lithography. ELSEVIER. *Biomaterials*. 20: 2363-2376.

[10] Chunlan Jiang, Rafael V. Davalos, and John C. Bischof. 2015. A Review Of Basic To Clinical Studies Of Irreversible Electroporation

[11] R. Sundrarajan T. Salameh, I.G. Camarillo and R. Raja Prabu, Arutselvan Natarajan and Kavitha Sankaranarayanan. 2013. Electroporation-Based Therapies for Cancer: From Basics to Clinical Applications. Elsevier, Aug 28, 2014-Medical – 352 pages. [Accessed on 06 November 2014].

[12] Antoni Ivorra and Boris Rubinsky. 2006. Impedance Analyzer for in vivo Electroporation Studies. *Proceedings of the 28th IEEE, EMBS Annual International Conference,* New York City, USA, Aug 30-Sept 3, 2006.

[13] Nobuyuki Tanaka, Hiroki Ota, Kazuhiro Fukumori, Masayuki Yamato, and Teruo Okano. 2013. Multiple Micro-Contact Printing Of Extra Cellular Matrix With Fine Alignment. *Micro-NanoMechatronics and Human Science (MHS), 2013 International Symposium* on japan. 10-13 Nov. IEEE. 1 – 2.

[14] M.I Maksud, M.S. Yusof, M. Mahadi. 2013. An Investigation of Parameter Effect on Microcontact Printing and Feasibility Study for Appication in Microelectronic and Biomedical. *The Biomedical Engineering International Conference (BMEiCON-2013)*.

[15] Sami Alom Ruiz and Christopher S. Chen. 2007. Microcontact Printing: A Tool To Pattern. *Soft Matter, The Royal Society Of Chemistry* 2007. 3: 1-11.

[16] James C. Weaver. 2000. Electroporation of Cells and Tissues. *IEEE Transaction on Plasma Science*. 28(1):. February 2000.

[17] Dong Qin, Younan Xia and George M. Whitesides. 2010. Soft Lithography For Micro- And Nanoscale Patterning. *Nature Protocols*. 5 (3): 491-502.

[18] Arjan P. Quist, Elisabeth Pavlovic, Sven Oscarsson. 2005. Recent Advances In Microcontact Printing. Review, *Anal Bioanal Chem* 381: 591-600.

[19] M. I Maksud, M. S. Yusof, M. Mahadi. 2013. A Study on Printed Multiple Solid Line by Combining Microcontact and Flexographic Printing Process for Microelectronic and Biomedical Application. *International Journal of Integrated Engineering*. 5(3): 36-39.

[20] A.L. Garner, J. Yang, N. Chen, J. Kolb, K. C. Lotfin, R. J. Swanson, S. Beebe, R. P. Joshi, and K. H. Schoenbach. Altering Dielectric Properties of Human Cancer Cells by Varying Electrical Pulse Durations. *Center of Biolelectrics, Old Dominion University*, Norfolk, Virginia 23510.

[21] Philip LeDuc, Emanuale Ostuni, George Whitesides, and Donald Ingber. Use of Micropatterned Adhesive Surfaces for Control of Cell Behavior. Chapter 19, Departments of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138. Department Of Pathology And Surgery Children's Hospital and Harvard Medical School, Boston, Massachusetts 02138.

[22] Y. Xia and G. M. Whitesides. 1998. Soft Lithography. angew. *Chemie Int*. Ed, 37(5): 550-575.

[23] Rafael V. Davalos, David M. Otten, Lluis M. Mir, and Boris Rubinsky. 2004. Electrical Impedance Tomography for Imaging Tissue Electroporation. *IEEE Transaction On Biomedical Engineering*. 51(5).

[24] Gorazd Pucihar, Member, IEEE, Jasna Krmelj, Matej Rebers˘ek, Tina Batista Napotnik, and Damijan Miklavcic. Equivalent Pulse Parameters for Electroporation. *IEEE Transaction on Biomedical Engineering*. 58(11) November 2011.

[25] J. P. Renault, A. Bernard, A. Bietsch, B. Michel, H. R. Bosshard, E. Delamarche, M. Kreiter, B. Hecht, and U. P. Wild. 2002. Fabricating Arrays Of Single Protein Molecules

On Glass Using Microcontact Printing. *America Chemical Society*.

[26] P. J. Canatella, M.M. Black, C. McKenna, J.F. Kan, J.A. Petros, M.R. Prausnitz. 1999. Electroporation Of Prostate Cancer Cells For Drug Delivery. *Proceedings Of The First Joint BMES/EMBS Conference Serving Humanity, Advancing Technology,* Oct 13-16 Atlanta, GA, USA.

[27] A.A. Sadiq, M.A.M. Mamman, M. Nazib Adon, N. B. Othman, M. Noh Dalimin, and M. Mahadi Abdul Jamil. 2015. An Overview: Investigation Of Electroporation And Sonoporation Techniques.

[28] Sonja A. Weber, Philipp A. Vonhoff, Francis J. Owens, J. Anthony Byrne and Eric T. McAdams. 2009. Development of Multi-Electrode Electrical Stimulation Device to improve Chronic Wound Healing. *31st Annual International Conference Of The IEEE EMBS Minneapolis*, Minnesota, USA, September 2-6. 2145-2148.

[29] Thomas F. Cronje, Paul T. Gaynor. 2013. High Voltage And Frequency Bipolar Pulse Generator Design For Electroporation-Based Cancer Therapy. AUPEC 2013, Hobart, TAS, Australia, 29 September – 3 October 2013.1-7.

[30] Paulo A. Garcia, John H. Rossmeisl, Jr., and Rafael V. Davalos. 2011. Electrical Conductivity Changes during Irreversible Electroporation Treatment of Brain Cancer. *33rd Annual international Conference of the IEEE EMBS Boston,* Massachusetts USA, August 30 – September 3. 739-742.

[31] Yoshiro ito. 1999. Surface Micropatterning to Regulate Cell Functions. Elsevier, *Biomaterials.* 20: 2333-2342.

[32] George D. O'Clock. 2014. A Multi-Scale Control System Model For Wound Healing Electrical Activity: Therapeutic Device/ Protocol Implications. *Engineering in Medicine and Biology Society (EMBC), 2014 36th Annual International Conference of the IEEE.* Chicago, IL. 26-30 Aug. 2014. 3021-3025.

[33] Ryoma Bise, Takeo Kanade, Zhaongzheng Yin, and Seung-il Huh. 2011. Automatic Cell tracking Applied to Analysis of Cell migration in Wound Healing Assay. *33rd Annual Int. Conference of IEEE EMBS Boston,* Massachusetts USA, August 30 – September 3. 6174-6179.

[34] Gan Yang, Haiyan Long, Jiang Wu, Hua Huang. 2008. A novel Bioreactor for Wound Healing Study. *International Conference on Biomedical Engineering and Informatics.*

[35] David Cukjati, Renata Karba, Stainslav Rebersek and Damijan Miklavcic. 2000. Measures of Wound healing rate. *Proceeding Of The 22th Annual EMBS International Conference.* Chicago IL July 23 –28.

[36] Olga Korostynska, Khalil Arshak, Edric Gill, and Arousian Arshak. 2007. Review Paper: Material and Techniques for In Vivo pH Monitoring. *IEEE Sensors Journal*. 8(1): January.

[37] David W. Jordan, Ronald M. Gilgenbach, Michael D. Uhler, Linda H. Gates, and Yue Ying Lau. 2004. Effects of Pulsed, High-Power Radiofrequency Radiation on Electroporation of Mamalian Cells. *IEEE Transaction on Plasma Science*. 32(4): August.

[38] Sukhendu B. Dev, Dietmar P. Rabussay, Georg Widera, and Gunter A. Hofmann. 2000. *Invited Paper, IEEE Transactions on Plasma Science.* 28(1): February.

[39] Min-Ji Kim, Taeyoon Kim, and Young-Ho Cho. 2011. A Cell Electroporation And Viability Monitoring Chip Using A Single Channel With Multiple Electric Field Zones. *Transducers'11*, Beijing, China, June 5-9. 2200-2202.

[40] Rafael V. Davalos, David M. Otten, Lluis M. Mir, and Boris Rubinsky. 2004. Electrical Impedance Tomography for imaging Tissue Electroporation. *IEEE Transactions on Biomedical Engineering.* 51(5): May.

[41] A.L. Vera Tizatl, L.I. Garay Jiménez, and S. A. Rodriguez Cuevas. 2013. 3D Model and Simulation of Electroporation Application on Healthy and Tumoral Breast Tissue. *10th International Conference on Electrical Engineering, Computing Science and Automatic Control (CCE)* Mexico City. 30 Sept – 4 Oct. 144 – 149.

[42] S. Rana, M. G. Tonnesen, X-D. Ren, R. A. Clark. 2007. Early Glycation of Critical Fibronectin domains inhibits human dermal fibroblast migration. *Bioengineering Conference, 2007. NEBC '07. IEEE 33rd Annual Northeast*. 10-11 March. 219 – 220.

[43] Karl H. Schoenbach. 2007. Bioelectrics – Using Nanosecond Pulsed Power Technology To Control Biological Cell Functions. Plasma Science, 2007. ICOPS 2007. *IEEE 34th International Conference* on 17-22 June.

[44] Lea Retelj, Gozard Pucihar, and Damijan Miklavčič. 2013. Electroporation of Intracellular Liposomes using Naosecond Electric Pulses- A theoretical Study. *IEEE Transaction on Biomedical Engineering,* 60(9). September.

[45] Gouping Guan, Lun Bai, Baoqi Zuo, Minzhong Li, Zhengyu Wu, Yonglin Li. 2009. Scaffolds Decorated by in vivo Environment Improve Cell Proliferation and Wound Healing. *Biomedical Engineering and Informatics, 2009. BMEI '09. 2nd International Conference* on 17-19 Oct.

[46] Tuhin Subhra Santra, Pen-Cheng Wang and Fang Gang Tseng. 2013. Electroporation Based Drug Delivery and Its Applications, Advances in Micro/Nano Electromechanical Systems and Fabrication Technologies, [Accessed on 6 November 2014].