Biomedical Engineering Applications: Cell Engineering

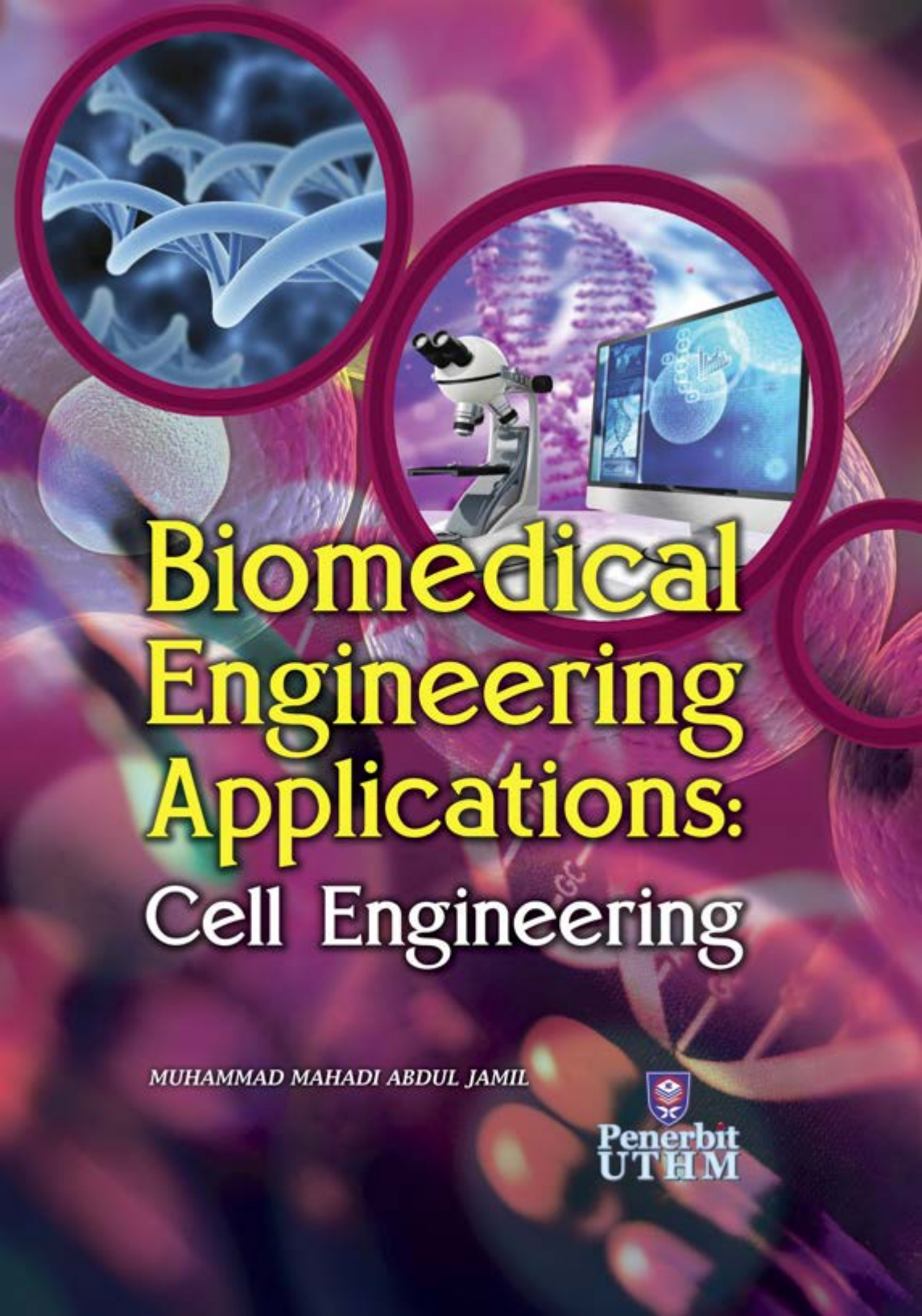
Author:
Muhammad Mahadi Abdul Jamil
Email:
mahadi@uthm.edu.my

Abstract: This book comprises of four chapters demonstrating the studies which involve cell culture with bioinstrumentation experimental work identifying the potential solutions for wound healing applications via exploitation of high voltage electric field or micro second pulse. The high voltage exposure on cells have shown interesting findings which gives us an idea on how to develop a dug free wound healing method in the nearest future.

The book also consists of two chapters that will present about the investigation of bone microstructures acquired from human samples. In this study there will be combination of bone histomorphology, imaging processing and computational method analysis thus also a new biomedical engineering application. Traditionally, the investigation of bone microstructures was performed by forensic officer by looking through microscope and their expert estimation by years of experience.

However, by having the engineers joining this investigation we could help the forensic officer to perform the analysis through computational method and automated analysis. Therefore the accomplishment of the six chapters will give an idea for the reader on what to expert in the field of research the so called “Cell Engineering”.

Keywords: Cell culture, human samples, computational method, automated analysis



Biomedical Engineering Applications:

Cell Engineering

MUHAMMAD MAHADI ABDUL JAMIL

Penerbit UTHM

Biomedical Engineering Applications: Cell Engineering

MUHAMMAD MAHADI ABDUL JAMIL

Penerbit UTHM
2017

Perpustakaan Negara Malaysia Cataloguing—in—Publication Data

Muhammad Mahadi Abdul Jamil
Biomedical Engineering Applications : Cell Engineering I
MUHAMMAD MAHADI ABDUL JAMIL.
ISBN 978-967-0764-80-1
1. Biomedical engineering. 2. Biotechnology.
3. Biological control systems. I. Title.
610.28

Published by:
Penerbit UTHM
Universiti Tun Hussein Onn Malaysia
86400 Parit Raja,
Batu Pahat, Johor
Tel: 07-453 8529
Fax: 07-453 6145

Website: http://penerbit.uthm.edu.my
E-mail: pt@uthm.edu.my
http://e-bookstore.uthm.edu.my

Penerbit UTHM is a member of
Majlis Penerbitan Ilmiah Malaysia
(MAPIM)

Printed by:
Percetakan Muafakat Jaya Sdn. Bhd.
No.6 Jalan Perdagangan 16,
Taman Universiti Industrial Park,
81300 Skudai, Johor
Tel: 07-520 6740
Fax: 07-520 6741

Dedication

This book is dedicated to the memory of my mother,
Aishah Bee.
Mother, only you who can inspire me.
You are my inspiration.
I miss you and the time we spent together.
The words I could not tell you, ***Mother I love you.***
May Allah grant you paradise.

Table of Contents

Preface *ix*

Acknowledgement *xi*

Chapter 1 **1**

ELECTROPORATION EFFECT ON HT29 CELL LINE PROLIFERATION RATE

Hassan Buhari Mamman, Muhammad Mahadi Abdul Jamil and Mohamad Nazib Adon

Chapter 2 **13**

ELECTROPORATION STUDY ON HT29 CELL LINE ATTACHMENT PROPERTIES

Hassan Buhari Mamman, Muhammad Mahadi Abdul Jamil and Mohamad Nazib Adon

Chapter 3 **25**

PULSE ELECTRIC FIELD EFFECT ON THE GROWTH OF HELA CELLS

Mohamed A. Milad Zaltum and Muhammad Mahadi Abdul Jamil

Chapter 4 **37**

PULSE DURATION EFFECT ON GROWTH RATE OF HELA CELLS

Mohamed A. Milad Zaltum, M. Mahadi Abdul Jamil and Nur Adilah Abd Rahman

Chapter 5 **47**

HUMAN BONE HISTOMORPHOLOGY STUDIES FOR GENDER IDENTIFICATION

Hadi Abdullah, Muhammad Mahadi Abdul Jamil and Faridah Mohd Nor

Chapter 6 **69**

HUMAN AGE PREDICTION AT DEATH VIA BONE MICROSTRUCTURES IMAGE PROCESSING

Ijaz Khan, Muhammad Mahadi Abdul Jamil and Faridah Mohd Nor

Index *87*

Preface

Biomedical engineering is an interesting field to study, research and explore. It's a merging of few disciplines into one focused specialization. In early days these fields were separated from each other such as science and engineering. Biomedical field merged from science discoveries and engineering as a technical and technological development. By combining these fields it becomes a multi-disciplinary research and it is termed as "Biomedical Engineering" as in the title of this book. Further, in this book we will show the studies performed that will potentially demonstrate the types of applications involved in this field. Other than that, the chapters included here will show the studies involved critically in the applications of "Cell Engineering" as in one of the important and focused area in Biomedical research.

This book comprises of six chapters in total. The first four chapters demonstrating the studies which involve cell culture with bioinstrumentation experimental work identifying the potential solutions for wound healing applications via exploitation of high voltage electric field or micro second pulse electric field. The high voltage exposure on cells have shown interesting findings which gives us an idea on how to develop a drug free wound healing method in the nearest future.

The other two chapters will demonstrate about the investigation of bone microstructures acquired from human samples. In this study there will be combination of bone histomorphology, image processing and computational method analysis thus also a new biomedical engineering application. Traditionally, the investigation of bone microstructures was performed by forensic officer by looking through microscope and their expert estimation by years of experience. However, by having the engineers joining this investigation we could help the forensic officer to perform the analysis through computational method and automated analysis.

Therefore the accomplishment of the six chapters will give an idea for the reader on what to expect in the field of research the so called "Cell Engineering".

Finally, I hope this small effort may have an important impact on contribution towards introducing Biomedical Engineering as an important academic & research field to specialize on particularly in Malaysia.

M. Mahadi Abdul Jamil

Acknowledgement

First and foremost, I would like to thank Almighty Allah for the blessings which enabled us to produce this book. I would like to express my sincere and deepest gratitude to the co-authors *Hassan Buhari Mamman, Mohamed Ahmed Milad Zaltum, Hadi Abdullah, & Ijaz Khan* for all the hard work where this edition was possible by their tremendous effort.

The support from the Faculty of Electrical and Electronic members, are also acknowledged.

Special dedication and gratitude to my parents, wife Azlina Hussin, and children Huzaifah, Hanzalah, Humairah, Muhammad Harith & Nur Muhammad for their patient, ongoing understanding, continuous support, encouragement and affection. Special appreciation also goes to my sisters Haajeeraah & Latefah, brother Latef & Bilal Saiboo and uncle Habibu Rahman all of them kept us going and made this journey a meaningful one.

In addition, I sincerely acknowledge the support and motivation provided by my respectful lecturers Ahmad Basri Zainal, Hisham Mat Husin, Mokhtar Jaafar, Rosli Abdullah, Dr Mansour Youseffi and Dr Morgan Denyer.

The full support from the Publisher Unit staff Dr Sapiee Jamel, Aida Maryani Abd Wahab, Yuniza Asri, Zuraidah Johari and others also acknowledged.

Last but not least I would like to thank all those people who have contributed directly or indirectly for the accomplishment of this book.

Chapter One

ELECTROPORATION EFFECT ON HT29 CELL LINE PROLIFERATION RATE

Hassan Buhari Mamman, Muhammad Mahadi Abdul Jamil and Mohamad Nazib Adon

ABSTRACT

Electroporation is a method of increasing cell membrane permeability and conductivity as a result of subjecting the cell to high intensity but a short electric field. Electroporation has been broadly used in medicine and biotechnology for cell fusion, electro-chemotherapy, gene-therapy, tumor cell ablation and sterilization of liquid food and water. In this study, the effect of electroporation on the cell size dynamics and growth of colon cells line HT29 are investigated. The primary aim is to see if electroporation can be used to increase the growth rate of cell lines that in turn can be used for wound healing application. With the help of BTX830 Electroporator, a 4mm cuvette and a voltage of 240V for duration of 500μs are used in electroporating the HT29 cells. The cells are then seeded in a flask and placed under appropriate physiological environment for observation. The electroporated cells are found to reach 96.1% confluence after 64 hours of seeding whereas the non-electroporated cells reached 76% confluence after 64 hours. Interestingly, both the electroporated cells and the non-electroporated cells attained a maximum length of 34.76μm±0.69 and 29.73μm±1.35 respectively after 24 hours of seeding. The study revealed that electroporation has an influence on the cell length and proliferation rate of HT29 cells line. This could be that the electric field facilitated the synthesis of the extracellular matrix protein and assisted the cell in taking more nutrients for growth and proliferation due to the pore formation during pulsing and the stimulation of the cell cytoskeleton restructuring.

BIBLIOGRAPHY

Adon, M. N., Dalimin, M. N., Jamil, M. M. A., Kassim, N. M., Hamdan, S. (2012). Study of the effect of microsecond pulsed electric fields on threshold area of HeLa cells. *IEEE EMBS International Conference on Biomedical Engineering and Sciences*. Pp 484-486.

Blackiston, D. J., McLaughlin, K. A., and Levin, M. (2009). Bioelectric controls of cell proferation: ion channels, membrane voltage and the cell cycle. *Cell cycle,* 8, pp3519-3528.

Hjouj, M., Last, D., Guez, D., Daniels, D. & Sharabi, S. (2012). MRI Study on Reversible and Irreversible Electroporation Induced Brain Barrier Disruption. *PLoS ONE* **7** (8). Pp 1-9.

Kotnik, T., Pucihar, G., & Miklavcic, D. (2010). Induced transmembrane voltage and its correlation with electroporation-mediated molecular transport. *The Journal of Membrane Biology*, ***236***(1). Pp 3–13.

Lin M. P., Marti G. P., Dieb R., Wang J., Qaiser R., Bande P., Duncan M. D., Harmon J. W. (2004). Electroporation improves transfection efficiency in rat wound healing. *Journal of the American College of surgeons. **199**(3).* Pp58-59.

Lin W., Cooper C., Camarillo I., Reece L. M., Clah L., Natararajan A., Campana L. G., Sundararajan R. (2014). The Effectiveneess of electroporation-based Nanocurcumin and Curcumin treatment on human breast cancer cells. *Proceeding ESA annual meeting on Electrostatics*. Pp 1-7.

Miklavcic, D., & Towhidi, L. (2010). Numerical study of the electroporation pulse shape effect on molecular uptake of biological cells. *Radiology and Oncology*, ***44***(1). Pp 34–41.

Milad Zaltum M. A., Adon M. N. & AbdulJamil M. M. (2013). Electroporation Effect on Growth of Hela Cells. Conference Proceedings, *IEEE Biomedical Engineering International Conference*. Pp 1-4.

Neal R. E., Singh R., Hatcher H. C., Kock N. D., Torti S. V., Davalos R. V. (2010). Treatment of breast cancer through the application of irreversible electroporation using a novel minimally invasive single electrode. *Breast Cancer Res Treat*, ***123***. Pp295-301.

Rebersek, M. and Miklavcic, D. (2011). Advantages and Disadvantages of Different Concepts of Electroporation Pulse Generation. *Journal Automatika*, ***52*** *(1).* Pp 12-19.

Suzuki, D. O. H., Ramos, A., Ribeiro, M. C. M., Cazarolli, L. H., Silva, F. R. M. B., Leite, L. D., & Marques, J. L. B. (2011). Theoretical and Experimental Analysis of Electroporated Membrane Conductance in Cell Suspension. *IEEE Transactions on Biomedical Engineering*, ***58***(12). Pp 3310–3318.

Talele, S., Gaynor, P., Cree, M. J., & van Ekeran, J. (2010). Modeling single cell electroporation with bipolar pulse parameters and dynamic pore radii. *Journal of Electrostatics*, ***68***(3). Pp 261–274.

Ziv R., Steinhardt Y., Pelled G., Gazit D., & Rubinsky B. (2009). Micro-electroporation of Mesenchymal stem cells with alternating electrical current pulses. *Biomed. Microdevices,* ***11***. Pp 95-101.

Zupanic, A., Kos, B., & Miklavcic, D. (2012). Treatment planning of electroporation-based medicalinterventions: electrochemotherapy, gene electrotransfer and irreversible electroporation. *Physics in Medicine and Biology*, ***57***(17). Pp 5425–5440.

Chapter Two

ELECTROPORATION STUDY ON HT29 CELL LINE ATTACHMENT PROPERTIES

Hassan Buhari Mamman, Muhammad Mahadi Abdul Jamil and Mohamad Nazib Adon

ABSTRACT

Electroporation as a process of exposing cell membrane to an external electric field is found not only to open pores in the cell membrane but also affect the cytoskeletal and signaling path ways of the cell. This chapter investigates the effect of electroporation on colon cell line, HT29 adhesion properties as a preliminary study on our desire to design a potential application of electroporation for wound healing. The study shows that when a single HT29 cell is electroporated with 600V/cm and 500μs pulse duration. The cell is attached to the monolayer 12 minutes after electroporation from floating state and spread to a distance of 38.98μm ± 1.03 in one hour. While non-electroporated cell line for control under the same condition attached to monolayer after 37 minutes from floating state and spread to a distance of 31.7μm ± 0.78 in one hour. The study shows that electroporated HT29 cell line attached faster and spread wider than non-electroporated HT29 cell line. This could be used to facilitate cell migration for wound healing application.

INTRODUCTION

Electroporation is a method of increasing cell membrane permeability and conductivity as a result of subjecting the cell to high intensity but short electric field. Electroporation has been broadly used in medicine and biotechnology for cell fusion, electro-chemotherapy, gene therapy, tumor cell ablation and sterilization of liquid food and water.

Even though, successes have been recorded in cell suspension culture, many applications in biology and biotechnology required cells that adhere onto a substrate. Adhesion of cells to each other and their

CONCLUSION

This study shows that the electroporation has a significant effect on HT29 cells line attachment. Thus, exposing HT29 cell line with 600V/cm electric field strength and 500μs pulse duration made the cells to attach faster to the monolayer for growth and development than Non-electroporated cells. Thus, it could be concluded that pulse electric field stimulated integrin, cadherin, filopodia and lamellipodia that are cell to cell and cell to substrate adhesion molecules. Therefore, the study could be useful to understand cell migration in wound healing application, since cell adhesion forms the basis of cell migration and other physiological processes.

BIBLIOGRAPHY

Arciero, J. C., Mi, Q., Branca, M. F., Hackam, D. J. & Swigon, A.(2011). Continuum model of collective cell migration in wound healing and colony expansion. *Biophysical Journal*, *100* pp535-543.

Chen, C. S., Alonso, J. L., Ostuni, E., Whitesides, G. M. & Ingber, D. E.(2003). Cell shape provides global control of focal adhesion assembly. *Biochemical and Biophysical research communication* 307 pp 355-361.

Hjouj, M., Last, D., Guez, D., Daniels, D. & Sharabi, S. (2012). MRI Study on Reversible and Irreversible Electroporation Induced Brain Barrier Disruption. *PLoS ONE* **7** (8). Pp 1-9.

Kotnik, T., Pucihar, G., & Miklavcic, D. (2010). Induced transmembrane voltage and its correlation with electroporation-mediated molecular transport. *The Journal of Membrane Biology*, ***236***(1). Pp 3–13.

Luna, S. M., Silva, S. S., Gomes, M. E., Mano, J. F. & Reis, R. L.(2011). Cell Adhesion and proliferation onto Chitosan-based membranes treated by Plasma surface modification. *Journal of biomaterials application*, 26 pp 101-116.

Maheshwari, G., Brown, G., Laufferenburger, D. A., Wells, A. & Griffith, L. G. (2000). Cell adhesion and motility depend on nanoscale RGD clustering. *Journal of cell science*, 113 pp1677-1686.

Miklavcic, D., & Towhidi, L. (2010). Numerical study of the electroporation pulse shape effect on molecular uptake of biological cells. *Radiology and Oncology*, ***44***(1). Pp 34–41.

Neal R. E., Singh R., Hatcher H. C., Kock N. D., Torti S. V., Davalos R. V. (2010). Treatment of breast cancer through the application of irreversible electroporation using a novel minimally invasive single electrode. *Breast Cancer Res Treat*, ***123***. Pp295-301.

Suzuki, D. O. H., Ramos, A., Ribeiro, M. C. M., Cazarolli, L. H., Silva, F. R. M. B., Leite, L. D., & Marques, J. L. B. (2011). Theoretical and Experimental Analysis of Electroporated Membrane Conductance in Cell Suspension. *IEEE Transactions on Biomedical Engineering*, ***58***(12). Pp 3310–3318.

Talele, S., Gaynor, P., Cree, M. J., & van Ekeran, J. (2010). Modeling single cell electroporation with bipolar pulse parameters and dynamic pore radii. *Journal of Electrostatics*, ***68***(3). Pp 261–274.

Thompson, O., Moore, C. J., Hussain, S. A., Kleino, L., Peckham, M., Hohenester, E., Ayscough, K. R., Saksela, K. & Winder, S. J. (2010). Modulation of cell spreading and cell substrate adhesion dynamics by dystroglycan. *Journal of cell science*, *123* pp118- 127.

Ziv R., Steinhardt Y., Pelled G., Gazit D., Rubinsky B. (2009). Micro-electroporation of Mesenchymal stem cells with alternating electrical current pulses. *Biomed. Microdevices,* ***11***. Pp 95-101.

Zupanic, A., Kos, B., & Miklavcic, D. (2012). Treatment planning of electroporation-based medical interventions: electrochemotherapy, gene electrotransfer and irreversible electroporation. *Physics in Medicine and Biology*, ***57***(17). Pp 5425–5440.

Chapter Three

PULSE ELECTRIC FIELD EFFECT ON THE GROWTH OF HELA CELLS

Mohamed A. Milad Zaltum and Muhammad Mahadi Abdul Jamil

ABSTRACT

Electroporation (EP) is a process of bio-physical effect on cells exposed to an external electrical field. It is gaining applications in medical treatments, especially to create pores through cell membrane and allow uptake of DNA into a cell. Therefore, in the theoretical evaluation of electroporation, transmembrane potential and characteristics of cell growth rate is the target of analysis. In this study we used cervical cancer cells (HeLa cells) as sample for electroporation, because HeLa cells is one of the most well-known cell lines, and easy to harvest continuously for large numbers of cells in order to perform in-vitro experimental tests. This study shows activity of HeLa cells exposed to high voltage of 2700V/cm with a pulse length of 10μs. The analysis shows that the HeLa cell growth rate with EP is 50% faster than normal growth rate.

INTRODUCTION

Electricity has been used in medicine for centuries, even long before the effect of electric and magnetic fields on biological tissue were in anyway understood. In the last decade modern science and technology have made the use of electromagnetic devices in medicine. Measurements of internal electric fields are taken routinely in diagnostics and electric stimulation of excitable tissues is used to sustain life, rehabilitate injuries and improve the quality of life in general. Electric fields can affect not only excitable tissues, such as muscles and nerves, but also non-excitable tissues, either thermally, by generating heat inside the tissue or by inducing structural changes down to cellular membranes. Numerous studies in the 1960s and 1970s have demonstrated that appropriate electric pulses can achieve electropermeabilization

many biomedical applications. The current findings give us extra dimensions to explore further down to cellular level which may contribute towards wound healing applications.

BIBLIOGRAPHY

Benedek, G., & Villars, F. (2000). *Physics with illustrative examples from medicine and biology:electricity and magnetism (second edition)*.

C. Schonenberger, A. Schlitz, A. Franco-Obregon, & Zenobi-Wong, M. (2011). Efficient electroporation of peptides into adherent cells: investigation of the role of mechano-growth factor in chondrocyte culture. *Biotechnology Letters, 33(5)*, 883-888.

Miklavcic D., & Puc, M. (2006). *Electroporation. Wiley Encyclopedia of Biomedical Engineering*, New York: John Wiley & Sons.

M. N. Adon, M. N. Dalimin, N. M. Kassim, & M. M. A. Jamil. (2012). Development of high voltage pulse inducement method for biological cell, in *Biomedical Engineering (ICoBE)*, 2012 International Conference, pp. 501-503.

Mohamad Nazib Adon, M. Noh Dalimin, Norazan Mohd Kassim, Sallehhuddin Hamdan, & M. M. Abdul Jamil. (2012). Study of Effect of Microsecond Pulsed Electric Fields on Threshold Area of HeLa Cells. *IEEE EMBS International Conference on Biomedical Engineering and Sciences*, pp. 484-486.

Neumann, E., Schaefer-Ridder M, Wang Y, Hofschneider PH. (1982). Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO J 1*: 841-845.

R. Reigada & M. L. Fernandez. (2011). Structure and electroporation of lipid bilayers: A Molecular Dynamics study, in *General Assembly and Scientific Symposium*, 2011 XXXth URSI, pp. 1-4.

Rowbottom, M., & Susskind, C. (1984). *Electricity and medicine*. San Francisco, CA, USA: San Francisco Press.

Rubinsky, B., Onik, G., & Mikus, P. (2007). Irreversible Electroporation: A New Ablation Modality -- Clinical Implications. Technology in *Cancer Research & Treatment, 6(1)*, 37-48.

Sugar IP, Neumann E. (1984). Stochastic model for electric field-induced membrane pores electroporation. *BiophysChem 19*: 211-225.

Tsong, T. (1991). Electroporation of cell membranes. *Biophysical Journal, 60(2)*, 297-306.

Usaj, M., Trontelj, K., Hudej, R., &Miklavcic D. (2009). Cell size dynamics and viability of cells exposed to hypotonic treatment and electroporation for electrofusion optimization. *Radiology and Oncology, 43(2).*

Zimmermann, U., Pilwat, G., & Riemann, F. (1974). Dielectric Breakdown of Cell Membranes. *Biophysical Journal, 14(11)*, 881-899.

Chapter Four

PULSE DURATION EFFECT ON GROWTH RATE OF HELA CELLS

Mohamed A. Milad Zaltum, Muhammad Mahadi Abdul Jamil and Nur Adilah Abd Rahman

ABSTRACT

Electroporation is the application of controlled direct current (DC) electrical pulses which are applied to living cells and tissues for a short duration of time. The pulse induce transmembrane potential which causes reversible break down of the cellular membrane. This action results in permeation or "pore formation" of the cell membrane which allows small molecules and large molecules to be introduced into the cell. During this process the cellular up take of the molecules continue until the pores close which can take milliseconds to minutes. In this study, we measured the relation between amplitude and duration in the Electroporation process. We have used cervical cancer cells (HeLa cells) to be the sample in this study. Cells are submitted to single pulse at constant field strength of 1kV/cm along with various pulse durations from 30μs to 600μs. Overall, the outcome of this research shows that pulse duration equal to100μs assisted HeLa cell growth rate tremendously in comparison to the pulse durations of 30μs, 300μs and 600μs.

INTRODUCTION

Biological effects of microsecond pulsed electric fields (μsPEF) have been intensively investigated over the last decade. Some researcher have been studying the biological effect of μsPEF and found that the transient increase in the permeability of cell membranes is used to introduce DNA or other molecules into cells. This phenomenon is potentially, the basis for many in vivo applications such as electro chemotherapy and gene therapy. However, it still lacks a comprehensive theoretical basis. This research involves in-vitro technique to evaluate specific cellular

CONCLUSION

This study observes experimentation of four pulse duration effect on growth rate of HeLa cell. Analysis shows that pulse duration plays a decisive role in increasing growth rate of HeLa cell. From this work it is concluded that, HeLa cell when exposed to 1kv/cm pulse with duration of 100μs the growth rate over time is greater when compared to growth rate of HeLa cell exposed with 30μs, 300μs and 600μs pulse duration. However, this research requires further investigation to identify the critical process of growth rate which might lead us on innovating potential applications. Finally, the current finding does give us a dimension to explore further down to the cellular level which may contribute towards wound healing applications.

BIBLIOGRAPHY

C. Schonenberger, A. Schlitz, A. Franco-Obregon, & M. Zenobi-Wong, M. (2011).). Efficient electroporation of peptides into adherent cells: investigation of the role of mechano-growth factor in chondrocyte culture. *Biotechnology Letters, 33(5)*, 883-888.

Khan, O., & El-Hag, A. (2011). Biological Cell Electroporation Using Nanosecond Electrical Pulses. *Journal Of Medical Imaging And Health Informatics, 1(3)*, 278-283.

Mitsutake, K., Satoh, A., Mine, S., Abe, K., Katsuki, S., & Akiyama, H. (2012). Effect of pulsing sequence of nanosecond pulsed electric fields on viability of HeLa S3 cells. *IEEE Transactions On Dielectrics And Electrical Insulation, 19(1)*, 337-342.

M. N. Adon, M. N. Dalimin, M. M. A. Jamil, & N. M. Kassim. (2012)., Development of high voltage pulse inducement method for biological cell, in *Biomedical Engineering (ICoBE)*, pp. 501-503.

M. Nazib Adon, M. Noh Dalimin, N. Mohd Kassim, & M. M. Abdul Jamil. (2011). *Microdosimetry Modeling Technique for Spherical Cell 5th Kuala Lumpur International Conference on Biomedical Engineering. vol. 35*, N. A. A. Osman, W. A. B. W. Abas, A. K. A. Wahab, and H.-N. Ting, Eds., ed: Springer Berlin Heidelberg, pp. 447-449.

Mohamed A. Zaltum, M. N. Adon, & M. Mahadi. Abdul Jamil. (2013). Electroporation Effect on Growth of HeLa Cell in *Biomedical Engineering (BMEiCON-2013).*

Mohamed A. Zaltum, M. N. Adon, Sallehuddin Hamdan, M. Noh Dalimin, & M. Mahadi Abdul Jamil. (2015). Investigation a Critical Selection of Pulse Duration Effect on Growth Rate of HeLa cells Proceedings of the *International Conference on BioSignal Analysis, Processing and System (ICBAPS 2015)*, Kuala Lampur, Malaysia.

M. Pech, A. Janitzky, J. Wendler, C. Strang, S. Blaschke, O. Dudeck, J. Ricke, & UB, Liehr.(2011)., *Irreversible Electroporation of Renal Cell Carcinoma: A First-in-Man Phase I Clinical Study, CardioVascular and Interventional Radiology, vol. 34*, pp. 132-138.

P. A. Garcia, R. E. Neal, M. B. Sano, 1. L. Robertson, & R. V. Davalos. (2011)., An experimental investigation of temperature changes during electroporation, in *General Assembly and Scientific Symposium, 2011* XXXth URSI, pp. 1-4.

Huo, R., Ma, Q., Wu, J., Chin-Nuke, K., Jing, Y., & Chen, J. et al. (2010). Noninvasive Electromagnetic Fields on Keratinocyte Growth and Migration. *Journal Of Surgical Research, 162(2)*, 299-307.

Joshi, R., & Hu, Q. (2010). Analysis of cell membrane permeabilization mechanics and pore shape due to ultrashort electrical pulsing. *Med Biol Eng Comput, 48(9)*, 837-844.

R. Reigada, & M. L. Fernandez. (2011). Structure and electroporation of lipid bilayers: A Molecular Dynamics study, in *General Assembly and Scientific Symposium, 2011* XXXth URSI, pp. 1-4.

Chapter Five

HUMAN BONE HISTOMORPHOLOGY STUDIES FOR GENDER IDENTIFICATION

Hadi Abdullah, Muhammad Mahadi Abdul Jamil and Faridah Mohd Nor

ABSTRACT

Bone consists of histological parameters or pattern that can be analyzed to determine different characteristics of a person or animal to which it belongs. Human-animal, gender, age, height and weight are some characteristics on which work has been done. Kerley in 1965 was the first to present microstructural computational method to determine age from human bone sample. This opened doors for research on age, gender and other characteristics determination. In literature main focus of research has been on age estimation. Gender estimation on the contrary has been a byproduct of age estimation or its left open to questions. Bone microstructural parameter differences change from people belonging to different regions and races and no parameters are set as standard for all human. At the same time, region and race specified age and gender estimation work has been done. This chapter discusses in detail the journey of gender estimation from bone histology. This includes selection of microstructural parameters used by different researchers, region and race of humans from which bones were taken and selection of the type of bone to analyze. Compilation of these researches giving concluding discussion on histological gender estimation from bone samples is also given. A proposed automated gender identification system which can identify gender from human bone sample using bone histomorphology is also given in this chapter. The proposed system is divided into two parts. In the first part bone samples are selected, manually analyzed and differences in microstructural parameters in males and females are observed. While in second part these observations are used to design computer aided automation system. Lastly the proposed system is concluded while giving its importance in multiple aspects.

researchers used different microstructural parameters in their research, depending on which of the parameters showed prominent differences in age and gender estimation. This chapter categorizes these parameters into two groups observed and derived. In each group some generally used parameters by researches in literature are given. After Kerley work in 1965, some researchers while working on age estimation claimed no gender differences in microstructural parameters. Based on this gender estimation became a byproduct of age estimation or was left with significant answers. This chapter compiles researches mainly focusing on gender estimation and which, while working on age estimation also estimated gender differentiating parameters. An automated design for gender estimation is discussed in details. This design is divided into two major parts. First part is biological aspect of research. In this part bone samples are prepared and manually observed. In second part image processing is introduced to automatically detect and observed microstructural parameters. Automated detection and observation of one of the parameter which is haversian canal is discussed for two samples. These two samples are ninety years old female and fifty six years old male Malaysian citizens. This observation agrees with literature related to gender estimation which is also discussed in this chapter.

BIBLIOGRAPHY

Bell, K. L., Loveridge, N., Reeve, J., Thomas, C. D., Feik, S. A., & Clement, J. G. (2001). Super-osteons (remodeling clusters) in the cortex of the femoral shaft: influence of age and gender. *Anat Rec, 264*(4), 378-38.

Burr, D. B., Ruff, C. B., & Thompson, D. D. (1990). Patterns of skeletal histologic change through time: comparison of an archaic native American population with modern populations. *Anat Rec, 226*(3), 307-313. doi: 10.1002/ar.1092260306

Ericksen, M. F. (1991). Histologic estimation of age at death using the anterior cortex of the femur. *Am J Phys Anthropol, 84*(2), 171-179.

Kerley, E. R. (1965). The microscopic determination of age in human bone. *American Journal of Physical Anthropology, 23*(2), 149-163. doi: 10.1002/ajpa.1330230215

Marieb, E. N. (2001). *Human anatomy & physiology*. San Francisco: Benjamin Cummings.

Meghan-Tómasita JúRi Cosgriff-Hernández, M. F. S. (2012). *Histomorphometric Estimation of Age at Death Using the Femoral Cortex: A Modification of Established Methods.* The Ohio State University.

Merriam-Webster Inc., w. M.-W. c.

Mulhern, D. M., & Van Gerven, D. P. (1997). Patterns of femoral bone remodeling dynamics in a Medieval Nubian population. *Am J Phys Anthropol, 104*(1), 133-146. doi: 10.1002/(sici)1096-8644(199709)104:1<133::aid-ajpa9>3.0.co;2-s.

Nor, F. M. (2009). *A comparative microscopic study of human and non-human long bone histology.* (PhD), University of Bradford.

Dreamstime, Osteon development and structure. Osteoblast, o., and osteoclast. http://www.dreamstime.com/.

Pfeiffer, S. (1998). Variability in osteon size in recent human populations. *Am J Phys Anthropol, 106*(2), 219-227. doi: 10.1002/(sici)1096-8644(199806)106:2<219::aid-ajpa8>3.0.co;2-k

Samson C, Branigan K. 1987. A new method of estimating age at death from fragmentary and weathered bone. In Bodington A, Garland AN, Janaway RC, editors. Death Decay and Reconstruction Approaches to Archaeology and Forensic Science. Manchester: Manchester University Press. p 101-108.

Singh, I. J., & Gunberg, D. L. (1970). Estimation of age at death in human males from quantitative histology of bone fragments. *American Journal of Physical Anthropology, 33*(3), 373-381. doi: 10.1002/ajpa.1330330311

Thompson, D. D. (1979). The core technique in the determination of age at death of skeletons. *J Forensic Sci, 24*(4), 902-915.

Thompson, D. D., & Gunness-Hey, M. (1981a). Bone mineral-osteon analysis of Yupik-inupiaq skeletons. *American Journal of Physical Anthropology, 55*(1), 1-7. doi: 10.1002/ajpa.1330550102

Thompson, D. D., & Gunness-Hey, M. (1981b). Bone mineral-osteon analysis of Yupik-Inupiaq skeletons. *Am J Phys Anthropol, 55*(1), 1-7. doi: 10.1002/ajpa.1330550102

Henry G. (1918). Anatomy of the Human Body.

http://www.bartleby.com/107/illus77.h, G. s. A. o. t. H.

Chapter Six

Human age prediction at death via bone microstructures image processing

Ijaz Khan, Muhammad Mahadi Abdul Jamil and Faridah Mohd Nor

ABSTRACT

The skeleton of human changes with increasing age due to change in bone density. Bone tissues have the capability to store the information of these variations which can be used to predict age from human remains after their death. This study discusses the process and factors that affect human skeleton with increasing age. The techniques to carry out human age estimation are discussed as well. Several methods for estimating human age at death using bone cross-sections have been proposed, which show that there is a strong correlation between increasing age and bone micro structures. These methods relied on manual calculation of various statistical measures from microscopic bone images and produced qualitative conclusions from their samples. Microscopic analysis of osteons, haversian canal and circumferential lamella were analyzed in these techniques to estimate human age. This chapter presents an automatic method of human age at death estimation using image processing and pattern recognition techniques. Bone samples are taken from ulna, radius, humerus, femur, tibia and fibula of Malaysian population. Ten different bone microstructures are selected for analysis in order to create regression equation for age estimation. Selected microstructure are extracted using image preprocessing and texture extraction algorithms. This chapter provides significant implications in the computation of fragmentary skeletal remains and forensic population samples for age estimation purpose.

BIBLIOGRAPHY

Alma,Y., Valds-Flores, M., Orozco, L., & Velzquez-Cruz, R. (2013). 'Molecular Aspects of Bone Remodeling', Topics In Osteoporosis.

Balthazard et Lebrun, (1911). *les canaux de havers de Io's humain aux differents ages*. An. Hyg. Publ. et Med, Leg., 114.

Cho, H., Stout, S.D., Madsen, R.W., and Streeter, M.A. (2002). 'Population-specific histological age-estimating method: a model for known African-American and European-American skeletal remains', Journal of forensic sciences. 47, (1), pp. 12-18.

Ericksen, M.F. (1991). Histologic estimation of age at death using the anterior cortex of the femur. *Am J Phys Anthropol. 84, (2)*, pp. 171-179.

Feng X, M. J. (2011). Disorders of Bone Remodeling, *Annu Rev Pathol (6)*, 121-145.

Kerley, E.R. (1965). The microscopic determination of age in human bone', *American Journal of Physical Anthropology, 23, (2)*, pp. 149-163.

Maat, G.J., Maes, A., Aarents, M.J., and Nagelkerke, N.J. (2006). Histological age prediction from the femur in a contemporary Dutch sample. The decrease of nonremodeled bone in the anterior cortex, *Journal of forensic sciences, 51(2)*, pp. 230-237.

Singh, I.J., and Gunberg, D.L. (1970). 'Estimation of age at death in human males from quantitative histology of bone fragments', *Am J Phys Anthropol 33(3),* pp. 373-381.

Stout, S.D. (1986). The use of bone histomorphometry in skeletal identification: the case of Francisco Pizarro. *Journal of forensic sciences, 31(1)*, pp. 296-300.

Thompson, D.D. (1979). The core technique in the determination of age at death of skeletons, *Journal of forensic sciences, 24(4)*, pp. 902-915.

Thompson, D.D. (1980). Age changes in bone mineralization, cortical thickness, and haversian canal area, *Calcif Tissue Int, 31(1)*, pp.

5-11.

Thompson, D.D. (1981). Microscopic determination of age at death in an autopsy series Journal of Forensic Science, 26(3), pp. 470-475.

Rutgers Website.

http://www.rci.rutgers.edu/~uzwiak/AnatPhys/APFallLect8.html