DEVELOPMENT OF AN ELECTRONIC AEROSOL ATOMISATION SYSTEM FOR GENERATING THREE-DIMENSIONAL (3D) CELLS IN MICROENCAPSULATIONS AND MICROTISSUES CHARACTERISATION

LEONG WAI YEAN

A thesis submitted in
fulfilment of the requirement for the award of the
Degree of Master of Electrical Engineering

Faculty of Electrical and Electronic Engineering
Universiti Tun Hussien Onn Malaysia

DECEMBER 2016

Special dedication with full gratitude on the guidance and encouragement to families who loved, especially my beloved father and mother and not forgotten to my supervisor that contributed ideas and opinions

ACKNOWLEDGEMENT

First, I would like to extend my deepest appreciation and heartfelt gratitude to my supervisor, Assoc. Prof. Dr. Soon Chin Fhong for her patience, tremendous support, and excellent guidance in terms of knowledge and continuous encouragement through my master studies, research, and thesis work, where ideas and supervision from her is very important in the completion of this project.

My special appreciations to my parents, family members and friends who has encourage, support, love, patience and understanding me throughout my involvement in this research project. Thank you for all the encouragement and affection given.

Finally thanks to my friends and any party involved directly or indirectly in this project which was conducted in Biosensor and Bioengineering Laboratory, Microelectronics and Nanotechnology – Shamsuddin Research Centre (MiNT-SRC), Faculty of Electrical and Electronic Engineering, Universiti Tun Hussein Onn Malaysia (UTHM).

We acknowledge Professor Cheong Sok Cheng from Cancer Research Malaysia for her kind contribution of oral squamous cell carcinoma (OSCC) cell line (ORL-48).

LIST OF ASSOCIATED PUBLICATIONS

Journal

1. Wai Yean Leong, Soon Chuan Wong, Kian Sek Tee, Sok Ching Cheong, Siew Hua Gan, Mansour Youseffi, Chin Fhong Soon, "*In vitro* growth of human keratinocytes and oral cancer cells into microtissues: an aerosol-based microencapsulation technique",*Biotechnology and Applied Biochemistry*. Impact factor: 1.429 (Q3, JCR, ISI Indexed). [In preparation]

2. Wai Yean Leong, Chin Fhong Soon, Soon Chuan Wong, Kian Sek Tee, "Development of an electronic aerosol system for generating microcapsules", *Journal Teknologi*, Volume 78, Issue 5-7, Pages: 79-85, May 2016. http://dx.doi.org/10.11113/jt.v78.8718 (Scopus Indexed).

ABSTRACT

Cell encapsulation is a micro technology widely applied in cell and tissue engineering, tissue transplantation and regenerative medicine. Various techniques had been developed for microencapsulation of cells but these techniques presented threat to the cells due to the harsh or chemical treatment applied. In this research, a simple and economic electronic aerosol atomisation system was proposed for producing calcium alginate microcapsules. The system was developed with the incorporation of a conventional syringe pump, a customised air pump and motor controller circuits. The microcapsules and 3D microtissues were biophysically characterised. For the output of the system, the microcapsules size slightly increased with the extrusion rates and decreased significantly with the airflow rates. At an extrusion rate of 20 µl/min and airflow rate of 0.3 l/min, microcapsules with a diameter ranging from 220 - 270 µm were generated. The polymerisation time for the microcapsules was approximately 10 minutes after the immersion in calcium chloride solutions. The microcapsules showed high porous surface structure in field emission-scanning electron microscopy (FE-SEM) imaging. Keratinocytes (HaCaT) and Oral Squamous Cell Carcinoma (ORL-48) cells at cell densities of 3×10^7 and 9×10^7 cells/ml, respectively were applied for encapsulation and successfully grew into microtissues after 16 days of culture. The fourier transform infrared (FTIR) spectroscopy of the 3D cells showed stretching in phosphate bond of Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) backbone, lipid and protein. The cells of HaCaT and ORL-48 microtissues were viable and they were characterised by different nucleus size. Replating experiment demonstrated that the cells in the microtissues could spread and proliferate in the culture dish. The electronic aerosol atomisation system developed in this work has successfully produced microcapsules with controllable size and applicable for growing microtissues. The microtissues produced are potentially a useful cell model for the study of cytochemicals.

ABSTRAK

Pengkapsulan sel adalah teknologi mikro digunakan secara meluas dalam bidang penyelidikan sel dan tisu, pemindahan tisu dan perubatan regeneratif. Pelbagai teknik telah dibangun untuk menghasilkan kapsul mikro untuk membalut sel tetapi memberi ancaman kepada sel disebabkan layanan kasar atau kimia semasa proses pengkapsulan. Dalam kajian ini, sistem pengabusan aerosol elektronik yang mudah dan ekonomi telah dicadang untuk menghasilkan kapsul mikro kalsium alginat. Sistem ini dibangunkan dengan penggabungan pam picagari konvensional, pam udara dan litar pengawal motor. Kapsul mikro dan tisu mikro telah dicirikan. Bagi output sistem, saiz kapsul mikro menunjukkan sedikit peningkatan dengan kadar penyemperitan dan menurun nyata sekali dengan kadar aliran udara. Pada 20 µl/min kadar penyemperitan dan 0.3 l/min kadar aliran udara, kapsul mikro dengan diameter 220 - 270 µm telah dihasilkan. Masa jangkaan polimerisasi kapsul mikro adalah 10 minit selepas rendam dalam larutan kalsium klorida. Kapsul mikro menunjukkan struktur permukaan yang berliang tinggi dalam pengimejan mikroskopi elektron imbasan-emisi medan (*FE-SEM)*. Sel *keratinocytes* (*HaCaT*) dan *Oral Squamous Cell Carcinoma* (*ORL-48*) pada kepadatan 3 $\times 10^7$ dan 9 $\times 10^7$ sel/ml telah digunakan untuk pengkapsulan dan berjaya tumbuh menjadi tisu mikro selepas 16 hari kultur. Inframerah transformasi Fourier (*FTIR*) bagi sel 3D menunjukkan peregangan ikatan fosfat dalam tulang belakang asid deoksibonukleik (*DNA*) dan asid ribonukleik (*RNA*), lipid dan protein. Sel tisu mikro *HaCaT* dan *ORL-48* hidup tetapi menunjukkan perbezaan dalam saiz nukleus. Eksperimen pemplatan semula menunjukkan bahawa sel-sel dalam tisu mikro boleh mengasingkan diri dan proliferat dalam bekas kultur. Sistem pengabusan aerosol elektronik dihasil dalam kerja ini berjaya menghasilkan saiz kapsul mikro yang boleh dikawal dan dapat digunakan untuk menumbuh tisu mikro. Tisu mikro yang dihasilkan adalah berpotensi untuk dijadikan model sel yang berguna untuk kajian sitokimia.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

2D	-	Two-Dimensional
3D	-	Three-Dimensional
$\propto$	-	Alpha
β	-	Beta
^{o}C	-	Degree Celsius
<	-	Lower Than
%	-	Percent
cells/ml	-	Cells per Milli Litre
cm	-	Centimeter
cm^2	-	Centimeter Square
f	-	Frequency
F	-	Force
cm^{-1}	-	Reciprocal Centimeter
kg/m^3	-	Kilo Gram per Cubic Meter
kV	-	Kilo Volt
l/min	-	Litre per Minute
µg/ml	-	Micro Gram per Milli Litre
µl	-	Micro Litre
µl/min	-	Micro Litre per Minute
µm	-	Micro Meter
µM	-	Micro Molar
mA	-	Milli Ampere
mg/l	-	Milli Gram per Litre
mg/ml	-	Milli Gram per Milli Litre
min	-	Minute

ml	-	Milli Litre
mm	-	Milli Meter
mM	-	Milli Molar
ms	-	Milli Second
ms^{-1}	-	Milli per Second
nm	-	Nano Meter
nM	-	Nano Molar
R^2	-	Coefficient of Determination
s	-	Second
units/ml	-	Units per Milli Litre
v	-	Velocity
V	-	Volume
A	-	Ampere
ARES	-	Advanced Routing and Editing Software
A-T	-	Adenine−Thymine
ATR	-	Attenuated Total Reflection
A-U	-	Adenine−Uracil
BD	-	Becton Dickinson
$CaCl_2$	-	Calcium Chloride
CLS	-	Cell Line Services
CO_2	-	Carbon Dioxide
DAPI	-	4', 6-Diamidino-2-Phenylindole Dihydrochloride
dc	-	Direct Current
DI	-	Deionised
DMEM	-	Dulbecco's Modified Eagle Medium
DNA	-	Deoxyribonucleic Acid
ECM	-	Extracellular Matrix
ER	-	Endoplasmic Reticulum
EthD-1	-	Ethidium Homodimer
ex/em	-	Excitation/Emission
FA	-	Focal Adhesion

FBS	-	Fetal Bovine Serum
FDA	-	Food and Drug Administration
FE-SEM	-	Field Emission-Scanning Electron Microscope
FTIR	-	Fourier Transform Infrared
G	-	Guluronate
HaCaT	-	Human Keratinocyte Cell Line
HBSS	-	Hank's Balanced Salt Solution
HTS	-	High-throughput Screening
Hz	-	Hertz
I-C	-	Hypoxanthine−Cytosine
ICF	-	Inertial Confinement Fusion
IL	-	Illinois
ISIS	-	Intelligent Schematic Input System
LABE	-	Low Angle Backscatter Imaging
LCD	-	Liquid Crystal Display
LED	-	Light Emitting Diode
LEI	-	Lower Secondary Electron Imaging
M	-	Mannuronate
MiNT-SRC	-	Microelectronics and Nanotechnology-Shamsuddin Research Centre
MO	-	Missouri
N	-	Newton
Na^+	-	Sodium
NaCl	-	Sodium Chloride
NIH	-	National Institutes of Health
ORL-48	-	Oral squamous cell carcinoma (OSCC) cell line
OSCC	-	Oral Squamous Cell Carcinoma
Pa	-	Pascal
PCB	-	Printed Circuit Board
P_d	-	Dynamic Pressure
PDMS	-	Polydimethylsiloxane

REFERENCES

[1] D. Antoni, H. Burckel, E. Josset, and G. Noel. Three-dimensional cell culture: a breakthrough in vivo. *International Journal of Molecular Sciences.* 2015. 16(3): 5517 - 5527.

[2] G. R. Souza, J. R. Molina, R. M. Raphael, M. G. Ozawa, D. J. Stark, C. S. Levin, L. F. Bronk, J. S. Ananta, J. Mandelin, M. M. Georgescu, J. A. Bankson, J. G. Gelovani, T. C. Killian, W. Arap, and R. Pasqualini. Three-dimensional tissue culture based on magnetic cell levitation. *Nature Nanotechnology.* 2010. 5(4): 291 - 296.

[3] R. Edmondson, J. J. Broglie, A. F. Adcock, and L. Yang. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. *Assay and Drug Development Technologies.* 2014. 12(4): 207 - 218.

[4] L. Kunz-Schughart, J. P. Freyer, F. Hofstaedter, and R. Ebner. The use of 3-D cultures for high throughput screening: the multicellular spheroid model. *Journal of Biomolecular Screening*. 2004. 9(4): 273 - 285.

[5] C. Soon, K. Thong, K. Tee, A. Ismail, M. Denyer, M. Ahmad, Y. Kong, P. Vyomesh, and S. Cheong. A scaffoldless technique for self-generation of three-dimensional keratinospheroids on liquid crystal surfaces. *Biotechnic & Histochemistry*. 2016. 91(4): 283 - 295.

[6] A. Kang, J. Park, J. Ju, G. S. Jeong, and S. H. Lee. Cell encapsulation via microtechnologies. *Biomaterials.* 2014. 35(9): 2651 - 2663.

[7] M. M. Stevens, H. F. Qanadilo, R. Langer, and V. Prasad Shastri. A rapid-curing alginate gel system: utility in periosteum-derived cartilage tissue engineering. *Biomaterials.* 2004. 25(5): 887 - 894.

[8] S. Sugiura, T. Oda, Y. Izumida, Y. Aoyagi, M. Satake, A. Ochiai, N. Ohkohchi, and M. Nakajima. Size control of calcium alginate beads containing living cells using micro-nozzle array. *Biomaterials.* 2005. 26(16): 3327 - 3331.

[9] E. L. Scheller, P. H. Krebsbach, and D. H. Kohn. Tissue engineering: state of the art in oral rehabilitation. *Journal of Oral Rehabilitation.* 2009. 36(5): 368 - 389.

[10] E. L. da Rocha, L. M. Porto, and C. R. Rambo. Nanotechnology meets 3D in vitro models: tissue engineered tumors and cancer therapies. *Materials Science & Engineering. C, Materials For Biological Applications.* 2014. 34: 270 - 279.

[11] C. S. Shin, B. Kwak, B. Han, and K. Park. Development of an in vitro 3D tumor model to study therapeutic efficiency of an anticancer drug. *Molecular Pharmaceutics.* 2013. 10(6): 2167 - 2175.

[12] T. M. Chang. Semipermeable Microcapsules. *Science.* 1964. 146(3643): 524 - 525.

[13] A. M. Sun. Microencapsulation of cells. Medical applications. *Annals of The New York Academy of Sciences.* 1997. 831: 271 - 279.

[14] L. Gasperini, J. F. Mano, and R. L. Reis. Natural polymers for the microencapsulation of cells. *Journal of The Royal Society Interface.* 2014. 11(100): 20140817.

[15] G. A. Paredes Juarez, M. Spasojevic, M. M. Faas, and P. de Vos. Immunological and technical considerations in application of alginate-based microencapsulation systems. *Frontiers in Bioengineering and Biotechnology.* 2014. 2: 26.

[16] V. Vaithilingam and B. E. Tuch. Islet transplantation and encapsulation: an update on recent developments. *The Review of Diabetic Studies.* 2011. 8(1): 51 - 67.

[17] J. M. Rabanel, X. Banquy, H. Zouaoui, M. Mokhtar, and P. Hildgen. Progress technology in microencapsulation methods for cell therapy. *Biotechnology Progress.* 2009. 25(4): 946 - 963.

[18] N. C. Hunt and L. M. Grover. Cell encapsulation using biopolymer gels for regenerative medicine. *Biotechnology Letters.* 2010. 32(6): 733 - 742.

[19] S. Sugiura, T. Oda, Y. Aoyagi, R. Matsuo, T. Enomoto, K. Matsumoto, T. Nakamura, M. Satake, A. Ochiai, N. Ohkohchi, and M. Nakajima.

Microfabricated airflow nozzle for microencapsulation of living cells into 150 micrometer microcapsules. *Biomedical Microdevices.* 2007. 9(1): 91 - 99.

[20] P. de Vos, C. G. van Hoogmoed, J. van Zanten, S. Netter, J. H. Strubbe, and H. J. Busscher. Long-term biocompatibility, chemistry, and function of microencapsulated pancreatic islets. *Biomaterials.* 2003. 24(2): 305 - 312.

[21] K. Y. Lee and D. J. Mooney. Alginate: properties and biomedical applications. *Progress In Polymer Science.* 2012. 37(1): 106 - 126.

[22] I. Ghidoni, T. Chlapanidas, M. Bucco, F. Crovato, M. Marazzi, D. Vigo, M. L. Torre, and M. Faustini. Alginate cell encapsulation: new advances in reproduction and cartilage regenerative medicine. *Cytotechnology.* 2008. 58(1): 49 - 56.

[23] P. de Vos, H. A. Lazarjani, D. Poncelet, and M. M. Faas. Polymers in cell encapsulation from an enveloped cell perspective. *Advanced Drug Delivery Reviews.* 2014. 67 - 68: 15 - 34.

[24] E. S. Chan, B. B. Lee, P. Ravindra, and D. Poncelet. Prediction models for shape and size of ca-alginate macrobeads produced through extrusion-dripping method. *Journal of Colloid and Interface Science.* 2009. 338(1): 63 - 72.

[25] S. Swioklo, P. Ding, A. W. Pacek, and C. J. Connon. Process parameters for the high-scale production of alginate-encapsulated stem cells for storage and distribution throughout the cell therapy supply chain. *Process Biochemistry.* 2016.

[26] A. Khademhosseini, G. Eng, J. Yeh, J. Fukuda, J. Blumling, R. Langer, and J. A. Burdick. Micromolding of photocrosslinkable hyaluronic acid for cell encapsulation and entrapment. *Journal of Biomedical Materials Research Part A.* 2006. 79(3): 522 - 532.

[27] W. G. Koh, A. Revzin, and M. V. Pishko. Poly(ethylene glycol) hydrogel microstructures encapsulating living cells. *Langmuir.* 2002. 18(7): 2459 - 2462.

[28] C. J. Martinez, J. W. Kim, C. Ye, I. Ortiz, A. C. Rowat, M. Marquez, and D. Weitz. A microfluidic approach to encapsulate living cells in uniform alginate hydrogel microparticles. *Macromolecular Bioscience.* 2012. 12(7): 946 - 951.

[29] K. S. Huang, M. K. Liu, C. H.Wu, Y. T. Yen, and Y. C. Lin. Calcium alginate microcapsule generation on a microfluidic system fabricated using the optical disk process. *Journal of Micromechanics and Microengineering*. 2007. 17(8): 1428 - 1434.

[30] Y. Hu, Q. Wang, J. Wang, J. Zhu, H. Wang, and Y. Yang. Shape controllable microgel particles prepared by microfluidic combining external ionic crosslinking. *Biomicrofluidics.* 2012. 6(2): 026502.

[31] W. Zhang and X. He. Encapsulation of living cells in small (approximately 100 microm) alginate microcapsules by electrostatic spraying: a parametric study. *Journal of Biomechanical Engineering.* 2009. 131(7): 074515.

[32] D. Lewinska, J. Bukowski, M. Kozuchowski, A. Kinasiewicz, and A. Werynski. Electrostatic microencapsulation of living cells. *Biocybernetics and Biomedical Engineering.* 2008. 28(2): 69 - 84.

[33] N. Li, X. X. Xu, G. W. Sun, X. Guo, Y. Liu, S. J. Wang, Y. Zhang, W. T. Yu, W. Wang, and X. J. Ma. The effect of electrostatic microencapsulation process on biological properties of tumour cells. *Journal of Microencapsulation.* 2013. 30(6): 530 - 537.

[34] L. Martin-Banderas, A. M. Ganan-Calvo, and M. Fernandez-Arevalo. Making Drops in Microencapsulation Processes. *Letters in Drug Design & Discovery.* 2010. 7(4): 300 - 309.

[35] J. H. Cui, J. S. Goh, S. Y. Park, P. H. Kim, and B. J. Le. Preparation and physical characterization of alginate microparticles using air atomization method. *Drug Development and Industrial Pharmacy.* 2001. 27(4): 309 - 319.

[36] E.P. Herrero, E.M. Mart´ın Del Valle, and M. A. Galan. Development of a new technology for the production of microcapsules based in atomization processes. *Chemical Engineering Journal.* 2006. 117(2): 137 - 142.

[37] M. Whelehan and I. W. Marison. Microencapsulation using vibrating technology. *Journal of Microencapsulation.* 2011. 28(8): 669 - 688.

[38] C. L. Herran and Y. Huang. Alginate microsphere fabrication using bipolar wave-based drop-on-demand jetting. *Journal of Manufacturing Processes.* 2012. 14(2): 98 - 106.

[39] C. Schwinger, S. Koch, U. Jahnz, P. Wittlich, N. G. Rainov, and J. Kressler. High throughput encapsulation of murine fibroblasts in alginate using the JetCutter technology. *Journal of Microencapsulation.* 2002. 19(3): 273 - 280.

[40] Q. Gao, Y. He, J.-z. Fu, J.-j. Qiu, and Y.-a. Jin. Fabrication of shape controllable alginate microparticles based on drop-on-demand jetting. *Journal of Sol-Gel Science and Technology.* 2015. 77(3): 610 - 619.

[41] A. Gautier, B. Carpentier, M. Dufresne, Q. Vu Dinh, P. Paullier, and C. Legallais. Impact of alginate type and bead diameter on mass transfers and the metabolic activities of encapsulated C3A cells in bioartificial liver applications. *European Cells & Materials.* 2011. 21: 94 - 106.

[42] J. Wan. Review Microfluidic-based synthesis of hydrogel particles for cell microencapsulation and cell-based drug delivery. *Polymers.* 2012. 4(2): 1084 - 1108.

[43] U. Prüsse, L. Bilancetti, M. Bučko, B. Bugarski, J. Bukowski, P. Gemeiner, D. Lewińska, V. Manojlovic, B. Massart, C. Nastruzzi, V. Nedovic, D. Poncelet, S. Siebenhaar, L. Tobler, A. Tosi, A. Vikartovsk á, and K.-D. Vorlop. Comparison of different technologies for alginate beads production. *Chemical Papers.* 2008. 62(4): 364 - 374.

[44] S. Tendulkar, S. H. Mirmalek-Sani, C. Childers, J. Saul, E. C. Opara, and M. K. Ramasubramanian. A three-dimensional microfluidic approach to scaling up microencapsulation of cells. *Biomedical Microdevices.* 2012. 14(3): 461 - 469.

[45] M. Whelehan and I. W. Marison. Microencapsulation by dripping and jet break up. *Bioencapsulation Innovations.* 2011. 1(1): 4 - 10.

[46] A. Dalmoro, A. A. Barba, and M. d'Amore. Analysis of size correlations for microdroplets produced by ultrasonic atomization. *The Scientific World Journal.* 2013. 2013: 7.

[47] Y. Zhang and M. L. Ma. *Microscale Technologies for Cell Engineering*. Springer International Publishing: Springer. 2016.

[48] D. Chicheportiche and G. Reach. In vitro kinetics of insulin release by microencapsulated rat islets: effect of the size of the microcapsules. *Diabetologia.* 1988. 31(1): 54 - 57.

[49] U. Pruesse, U. Jahnz, P. Wittlich, and K. D. Vorlop. Scale-up of the JetCutter technology. *Chemistry & Industry.* 2003. 12: 636 - 641.

[50] C. Heinzen, A. Berger, and I. Marison. Use of vibration technology for jet break-up for encapsulation of cells and liquids in monodisperse microcapsules. In: V. Nedović and R. Willaert. *Fundamentals of Cell Immobilisation Biotechnology*. Dordrecht: Springer Netherlands. 2004. 257 - 275.

[51] M. A. Neves, I. Kobayashi, and M. Nakajima. Development of microchannel emulsification technology for monodispersed soybean and olive oil-in-water emulsions. *Journal of Arid Land Studies*. 2009. 19(1): 97 - 100.

[52] C. Kim, K. S. Lee, Y. E. Kim, K. J. Lee, S. H. Lee, T. S. Kim, and J. Y. Kang. Rapid exchange of oil-phase in microencapsulation chip to enhance cell viability. *Lab On A Chip.* 2009. 9(9): 1294 - 1297.

[53] A. Sohail, M. S. Turner, A. Coombes, T. Bostrom, and B. Bhandari. Survivability of probiotics encapsulated in alginate gel microbeads using a novel impinging aerosols method. *International Journal of Food Microbiology.* 2011. 145(1): 162 - 168.

[54] S. Ahn, H. Lee, L. J. Bonassar, and G. Kim. Cells (MC3T3-E1)-laden alginate scaffolds fabricated by a modified solid-freeform fabrication process supplemented with an aerosol spraying. *Biomacromolecules.* 2012. 13(9): 2997 - 3003.

[55] S. Ahn and G. Kim. Cell-encapsulating alginate microsized beads using an air-assisted atomization process to obtain a cell-laden hybrid scaffold. *Journal of Materials Chemistry B.* 2015. 3(47): 9132 - 9139.

[56] S. Hamid, K. P. Lim, R. B. Zain, S. M. Ismail, S. H. Lau, W. M. Mustafa, M. T. Abraham, N. A. Nam, S. H. Teo, and S. C. Cheong. Establishment and characterization of Asian oral cancer cell lines as in vitro models to study a disease prevalent in Asia. *International Journal of Molecular Medicine*. 2007. 19(3): 453 - 460.

[57] C. Scully and S. Porter. ABC of oral health. Oral cancer. *BMJ - British Medical Journal.* 2000. 321(7253): 97 - 100.

[58] W. Chen, J. H. Kim, D. Zhang, K. H. Lee, G. A. Cangelosi, S. D. Soelberg, C. E. Furlong, J. H. Chung, and A. Q. Shen. Microfluidic one-step synthesis of alginate microspheres immobilized with antibodies. *Journal of The Royal Society Interface.* 2013. 10(88): 20130566.

[59] E. Bianconi, A. Piovesan, F. Facchin, A. Beraudi, R. Casadei, F. Frabetti, L. Vitale, M. C. Pelleri, S. Tassani, F. Piva, S. Perez-Amodio, P. Strippoli, and S. Canaider. An estimation of the number of cells in the human body. *Annals of Human Biology.* 2013. 40(6): 463 - 471.

[60] L. Sherwood. *Fundamentals of Human Physiology*. 4th Ed. USA: Brooks/Cole Cengage Learning. 2012.

[61] M. Wilson. *Microbial inhabitants of Humans: Their Ecology and Role in Health and Disease*. 1st Ed. United Kingdom: Cambridge University Press. 2005.

[62] J. McDowell. *Encyclopedia of Human Body Systems Volume 1*. USA: Greenwood. 2010.

[63] M. Chiquet, M. Matthisson, M. Koch, M. Tannheimer, and R. Chiquet-Ehrismann. Regulation of extracellular matrix synthesis by mechanical stress. *Biochemistry and Cell Biology-Biochimie ET Biologie Cellulaire.* 1996. 74(6): 737 - 744.

[64] R. Londono, V. S. Gorantla, and S. F. Badylak. Emerging implications for extracellular matrix-based technologies in vascularized composite allotransplantation. *Stem Cells International.* 2016. 2016(1541823): 16.

[65] M. Fujita, D. C. Spray, H. Choi, J. Saez, D. M. Jefferson, E. Hertzberg, L. C. Rosenberg, and L. M. Reid. Extracellular matrix regulation of cell-cell communication and tissue-specific gene expression in primary liver cultures. *Progress in Clinical and Biological Research.* 1986. 226(1): 333 - 360.

[66] C. Frantz, K. M. Stewart, and V. M. Weaver. The extracellular matrix at a glance. *Journal of Cell Science.* 2010. 123(24): 4195 - 4200.

[67] S. H. Kim, J. Turnbull, and S. Guimond. Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. *The Journal of Endocrinology.* 2011. 209(2): 139 - 151.

[68] M. P. Sheetz, D. P. Felsenfeld, and C. G. Galbraith. Cell migration: regulation of force on extracellular-matrix-integrin complexes. *Trends in Cell Biology.* 1998. 8(2): 51 - 54.

[69] K. Wolf, M. Te Lindert, M. Krause, S. Alexander, J. Te Riet, A. L. Willis, R. M. Hoffman, C. G. Figdor, S. J. Weiss, and P. Friedl. Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force. *The Journal of Cell Biology.* 2013. 201(7): 1069 - 1084.

[70] B. M. Gumbiner. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell.* 1996. 84(3): 345 - 357.

[71] H. Truong and E. H. Danen. Integrin switching modulates adhesion dynamics and cell migration. *Cell Adhesion & Migration.* 2009. 3(2): 179 - 181.

[72] J. T. Parsons, A. R. Horwitz, and M. A. Schwartz. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nature Reviews Molecular Cell Biology.* 2010. 11(9): 633 - 643.

[73] M. Nagano, D. Hoshino, N. Koshikawa, T. Akizawa, and M. Seiki. Turnover of focal adhesions and cancer cell migration. *International Journal of Cell Biology.* 2012. 2012(310616): 10.

[74] V. Petit and J. P. Thiery. Focal adhesions: structure and dynamics. *Biology of The Cell.* 2000. 92(7): 477 - 494.

[75] B. Geiger, A. Bershadsky, R. Pankov, and K. M. Yamada. Transmembrane crosstalk between the extracellular matrix--cytoskeleton crosstalk. *Nature Reviews. Molecular Cell Biology.* 2001. 2(11): 793 - 805.

[76] B. H. Luo, C. V. Carman, and T. A. Springer. Structural basis of integrin regulation and signaling. *Annual Review of Immunology.* 2007. 25: 619 - 647.

[77] E. Goksoy, Y. Q. Ma, X. Wang, X. Kong, D. Perera, E. F. Plow, and J. Qin. Structural basis for the autoinhibition of talin in regulating integrin activation. *Molecular Cell.* 2008. 31(1): 124 - 133.

[78] J. D. Humphries, P. Wang, C. Streuli, B. Geiger, M. J. Humphries, and C. Ballestrem. Vinculin controls focal adhesion formation by direct interactions with talin and actin. *The Journal of Cell Biology.* 2007. 179(5): 1043 - 1057.

[79] W. R. Legant, C. S. Chen, and V. Vogel. Force-induced fibronectin assembly and matrix remodeling in a 3D microtissue model of tissue morphogenesis. *Integrative Biology : Quantitative Biosciences From Nano To Macro*. 2012. 4(10): 1164 - 1174.

[80] J. A. Hickman, R. Graeser, R. de Hoogt, S. Vidic, C. Brito, M. Gutekunst, H. van der Kuip, and I. P. Consortium. Three-dimensional models of cancer for pharmacology and cancer cell biology: capturing tumor complexity in vitro/ex vivo. *Biotechnology Journal*. 2014. 9(9): 1115 - 1128.

[81] C. L. Simpson, D. M. Patel, and K. J. Green. Deconstructing the skin: cytoarchitectural determinants of epidermal morphogenesis. *Nature Reviews. Molecular Cell Biology*. 2011. 12(9): 565 - 580.

[82] A. Pessina, A. Raimondi, A. Cerri, M. Piccirillo, M. G. Neri, C. Croera, P. Foti, and E. Berti. High sensitivity of human epidermal keratinocytes (HaCaT) to topoisomerase inhibitors. *Cell Proliferation*. 2001. 34(4): 243 - 252.

[83] P. Boukamp, R. T. Petrussevska, D. Breitkreutz, J. Hornung, A. Markham, and N. E. Fusenig. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *The Journal of Cell Biology*. 1988. 106(3): 761 - 771.

[84] A. F. Deyrieux and V. G. Wilson. In vitro culture conditions to study keratinocyte differentiation using the HaCaT cell line. *Cytotechnology*. 2007. 54(2): 77 - 83.

[85] V. G. Wilson. Growth and differentiation of HaCaT keratinocytes. *Methods In Molecular Biology*. 2014. 1195: 33 - 41.

[86] V. M. Schoop, N. Mirancea, and N. E. Fusenig. Epidermal organization and differentiation of HaCaT keratinocytes in organotypic coculture with human dermal fibroblasts. *The Journal of Investigative Dermatology*. 1999. 112(3): 343 - 353.

[87] N. Schurer, A. Kohne, V. Schliep, K. Barlag, and G. Goerz. Lipid composition and synthesis of HaCaT cells, an immortalized human keratinocyte line, in comparison with normal human adult keratinocytes. *Experimental Dermatology*. 1993. 2(4): 179 - 185.

[88] E. Boelsma, M. C. H. Verhoeven, and M. Ponec. Reconstruction of a human skin equivalent using a spontaneously transformed keratinocyte cell line (HaCaT). *Journal of Investigative Dermatology*. 1999. 112(4): 489 - 498.

[89] K. M. Yamada and E. Cukierman. Modeling tissue morphogenesis and cancer in 3D. *Cell.* 2007. 130(4): 601 - 610.

[90] P. B. Sugerman and N. W. Savage. Current concepts in oral cancer. *Australian Dental Journal.* 1999. 44(3): 147 - 156.

[91] R. B. Zain and N. Gahzali. A review of epidemiological studies of oral cancer and precance in Malaysia. *Annals of Dentistry*. 2001. 8(1): 50 - 56.

[92] K. R. Loeb and L. A. Loeb. Significance of multiple mutations in cancer. *Carcinogenesis.* 2000. 21(3): 379 - 385.

[93] J. Califano, P. van der Riet, W. Westra, H. Nawroz, G. Clayman, S. Piantadosi, R. Corio, D. Lee, B. Greenberg, W. Koch, and D. Sidransky. Genetic progression model for head and neck cancer: implications for field cancerization. *Cancer Research.* 1996. 56(11): 2488 - 2492.

[94] K. M. Yamada and E. Cukierman. Modeling tissue morphogenesis and cancer in 3D. *Cell*. 2007. 130(4): 601 - 610.

[95] W. Fayad, L. Rickardson, C. Haglund, M. H. Olofsson, P. D'Arcy, R. Larsson, S. Linder, and M. Fryknäs. Identification of agents that induce apoptosis of multicellular tumour spheroids: enrichment for mitotic inhibitors with hydrophobic properties. *Chemical Biology & Drug Design.* 2011. 78(4): 547 - 557.

[96] F. Hirschhaeuser, H. Menne, C. Dittfeld, J. West, W. Mueller-Klieser, and L. A. Kunz-Schughart. Multicellular tumor spheroids: An underestimated tool is catching up again. *Journal of Biotechnology.* 2010. 148(1): 3 - 15.

[97] S. C. Wong, C. F. Soon, W. Y. Leong, and K. S. Tee. Flicking technique for microencapsulation of cells in calcium alginate leading to the microtissue formation. *Journal of Microencapsulation.* 2016. 33(2): 162 - 171.

[98] M. M. Vantangoli, S. J. Madnick, S. M. Huse, P. Weston, and K. Boekelheide. MCF-7 human breast cancer cells form differentiated microtissues in scaffold-free hydrogels. *PLoS One.* 2015. 10(8): e0135426.

[99] V. S. Nirmalanandhan, A. Duren, P. Hendricks, G. Vielhauer, and G. S. Sittampalam. Activity of anticancer agents in a three-dimensional cell culture model. *Assay and Drug Development Technologies.* 2010. 8(5): 581 - 590.

[100] C. Godugu, A. R. Patel, U. Desai, T. Andey, A. Sams, and M. Singh. AlgiMatrix™ based 3D cell culture system as an in-vitro tumor model for anticancer studies. *PLoS One.* 2013. 8(1): e53708.

[101] J. W. Haycock. 3D cell culture: a review of current approaches and techniques. *Methods in Molecular Biology.* 2011. 695: 1 - 15.

[102] M. Rimann and U. Graf-Hausner. Synthetic 3D multicellular systems for drug development. *Current Opinion in Biotechnology.* 2012. 23(5): 803 - 809.

[103] A. P. Napolitano, D. M. Dean, A. J. Man, J. Youssef, D. N. Ho, A. P. Rago, M. P. Lech, and J. R. Morgan. Scaffold-free three-dimensional cell culture utilizing micromolded nonadhesive hydrogels. *BioTechniques.* 2007. 43(4): 494 - 500.

[104] T. M. Achilli, J. Meyer, and J. R. Morgan. Advances in the formation, use and understanding of multi-cellular spheroids. *Expert Opinion on Biological Therapy.* 2012. 12(10): 1347 - 1360.

[105] E. Gevaert, L. Dolle, T. Billiet, P. Dubruel, L. van Grunsven, A. van Apeldoorn, and R. Cornelissen. High throughput micro-well generation of hepatocyte micro-aggregates for tissue engineering. *PLoS One.* 2014. 9(8): e105171.

[106] M. Drewitz, M. Helbling, N. Fried, M. Bieri, W. Moritz, J. Lichtenberg, and J. M. Kelm. Towards automated production and drug sensitivity testing using scaffold-free spherical tumor microtissues. *Biotechnology Journal.* 2011. 6(12): 1488 - 1496.

[107] P. Occhetta, M. Centola, B. Tonnarelli, A. Redaelli, I. Martin, and M. Rasponi. High-throughput microfluidic platform for 3d cultures of mesenchymal stem cells, towards engineering developmental processes. *Scientific Reports.* 2015. 5: 10288.

[108] J. L. Wilson and T. C. McDevitt. Stem cell microencapsulation for phenotypic control, bioprocessing, and transplantation. *Biotechnology and Bioengineering.* 2013. 110(3): 667 - 682.

[109] J. Schweicher, C. Nyitray, and T. A. Desai. Membranes to achieve immunoprotection of transplanted islets. *Frontiers in Bioscience.* 2014. 19: 49 - 76.

[110] R. Dubey, T. C. Shami, and K. U. B. Rao. Microencapsulation technology and applications. *Defence Science Journal.* 2009. 59(1): 82 - 95.

[111] M. N. Singh, K. S. Hemant, M. Ram, and H. G. Shivakumar. Microencapsulation: A promising technique for controlled drug delivery. *Research in Pharmaceutical Sciences.* 2010. 5(2): 65 - 77.

[112] G. J. Wang, L. Y. Chu, M. Y. Zhou, and W. M. Chen. Effects of preparation conditions on the microstructure of porous microcapsule membranes with straight open pores. *Journal of Membrane Science.* 2006. 284(1-2): 301 - 312.

[113] S. V. Bhujbal, G. A. Paredes-Juarez, S. P. Niclou, and P. de Vos. Factors influencing the mechanical stability of alginate beads applicable for immunoisolation of mammalian cells. *Journal of the Mechanical Behavior of Biomedical Materials.* 2014. 37: 196 - 208.

[114] H. B. Scher, M. Rodson, and K. S. Lee. Microencapsulation of pesticides by interfacial polymerization utilizing isocyanate or aminoplast chemistry. *Journal of Pesticide Science*. 1998. 54(4): 394 - 400.

[115] G. Bingham, R. V. Gunning, K. Gorman, L. M. Field, and G. D. Moores. Temporal synergism by microencapsulation of piperonyl butoxide and alpha-cypermethrin overcomes insecticide resistance in crop pests. *Pest Management Science.* 2007. 63(3): 276 - 281.

[116] X. L. Gu, X. Zhu, X. Z. Kong, and Y. Tan. Comparisons of simple and complex coacervations for preparation of sprayable insect sex pheromone microcapsules and release control of the encapsulated pheromone molecule. *Journal of Microencapsulation.* 2010. 27(4): 355 - 364.

[117] V. D. Prajapati, G. K. Jani, and J. R. Kapadia. Current knowledge on biodegradable microspheres in drug delivery. *Expert Opinion on Drug Delivery.* 2015: 1 - 17.

[118] D. H. Kim, J. Chen, R. A. Omary, and A. C. Larson. MRI visible drug eluting magnetic microspheres for transcatheter intra-arterial delivery to liver tumors. *Theranostics.* 2015. 5(5): 477 - 488.

[119] C. A. Garcia-Gonzalez, M. Jin, J. Gerth, C. Alvarez-Lorenzo, and I. Smirnova. Polysaccharide-based aerogel microspheres for oral drug delivery. *Carbohydrate Polymers.* 2015. 117: 797 - 806.

[120] Q. Li, G. Zhou, X. Yu, T. Wang, Y. Xi, and Z. Tang. Porous deproteinized bovine bone scaffold with three-dimensional localized drug delivery system using chitosan microspheres. *Biomedical Engineering Online.* 2015. 14(1): 33.

[121] R. Rastogi, Y. Sultana, M. Aqil, A. Ali, S. Kumar, K. Chuttani, and A. K. Mishra. Alginate microspheres of isoniazid for oral sustained drug delivery. *International Journal of Pharmaceutics.* 2007. 334(1-2): 71 - 77.

[122] B. M. Estevinho, F. A. Rocha, L. M. Santos, and M. A. Alves. Using water-soluble chitosan for flavour microencapsulation in food industry. *Journal of Microencapsulation.* 2013. 30(6): 571 - 579.

[123] I. H. Kim, J. Han, J. H. Na, P. S. Chang, M. S. Chung, K. H. Park, and S. C. Min. Insect-resistant food packaging film development using cinnamon oil and microencapsulation technologies. *Journal of Food Science.* 2013. 78(2): 229 - 237.

[124] M. I. Dias, I. C. Ferreira, and M. F. Barreiro. Microencapsulation of bioactives for food applications. *Food & Function.* 2015. 6(4): 1035 - 1052.

[125] F. Nazzaro, P. Orlando, F. Fratianni, and R. Coppola. Microencapsulation in food science and biotechnology. *Current Opinion in Biotechnology.* 2012. 23(2): 182 - 186.

[126] S. S. Kuang, J. C. Oliveira, and A. M. Crean. Microencapsulation as a tool for incorporating bioactive ingredients into food. *Critical Reviews in Food Science and Nutrition.* 2010. 50(10): 951 - 968.

[127] K. K. Mishra, R. K. Khardekar, R. Singh, and H. C. Pant. Fabrication of polystyrene hollow microspheres as laser fudion targets by optimized density matched emulsion technique and characterization. *Pramana-Journal of Physics.* 2002. 59(1): 113 - 131.

[128] H. S. Park, O. A. Hurricane, D. A. Callahan, D. T. Casey, E. L. Dewald, T. R. Dittrich, T. Doppner, D. E. Hinkel, L. F. Berzak Hopkins, S. Le Pape, T. Ma, P. K. Patel, B. A. Remington, H. F. Robey, J. D. Salmonson, and J. L. Kline. High-adiabat high-foot inertial confinement fusion implosion experiments on the national ignition facility. *Physical Review Letters.* 2014. 112(5): 055001.

[129] J. Burgain, C. Gaiani, M. Linder, and J. Scher. Review: Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering.* 2011. 104(4): 467 - 483.

[130] G. K. Gbassi and T. Vandamme. Probiotic encapsulation technology: from microencapsulation to release into the gut. *Pharmaceutics.* 2012. 4(1): 149 - 163.

[131] H. K. Solanki, D. D. Pawar, D. A. Shah, V. D. Prajapati, G. K. Jani, A. M. Mulla, and P. M. Thakar. Development of microencapsulation delivery system for long-term preservation of probiotics as biotherapeutics agent. *BioMed Research International.* 2013. 2013(620719): 21.

[132] U. Prube, U. Jahnz, P. Wittlich, J. Breford, and K. D. Vorlop. Bead production with JetCutting and rotating disc/nozzle technologies. *Landbauforschung.* 2002. 241: 1 - 10.

[133] K. Kailasapathy. Microencapsulation of probiotic bacteria: technology and potential applications. *Current Issues in Intestinal Microbiology.* 2002. 3(2): 39 - 48.

[134] U. Prüsse, B. Fox, M. Kirchhoff, F. Bruske, J. Breford, and K. D. Vorlop. New process (Jet Cutting Method) for the production of spherical beads from highly viscous polymer solutions. *Chemical Engineering & Technology.* 1998. 21(1): 29 - 33.

[135] T. A. Horbett, J. J. Waldburger, B. D. Ratner, and A. S. Hoffman. Cell adhesion to a series of hydrophilic-hydrophobic copolymers studied with a spinning disc apparatus. *Journal of Biomedical Materials Research.* 1988. 22(5): 383 - 404.

[136] R. Dorati, I. Genta, T. Modena, and B. Conti. Microencapsulation of a hydrophilic model molecule through vibration nozzle and emulsion phase inversion technologies. *Journal of Microencapsulation.* 2013. 30(6): 559 - 570.

[137] Y. Zhang and D. Rochefort. Comparison of emulsion and vibration nozzle methods for microencapsulation of laccase and glucose oxidase by interfacial reticulation of poly(ethyleneimine). *Journal of Microencapsulation.* 2010. 27(8): 703 - 713.

[138] A. Picot, and C. Lacroix. Production of multiphase water-insoluble microcapsules for cell encapsulation using an emulsification/spray-drying technology. *Journal of Food Science.* 2003. 68(9): 2693 - 2700.

[139] L. Liu, F. Wu, X. J. Ju, R. Xie, W. Wang, C. H. Niu, and L. Y. Chu. Preparation of monodisperse calcium alginate microcapsules via internal gelation in microfluidic-generated double emulsions. *Journal of Colloid and Interface Science.* 2013. 404: 85 - 90.

[140] T. A. B. Bressel, A. H. Paz, G. Baldo, E. O. C. Lima, U. Matte, and M. L. Saraiva-Pereira. An effective device for generating alginate microcapsules. *Genetics and Molecular Biology.* 2008. 31(1): 136 - 140.

[141] W. Y. Leong, C. F. Soon, S. C. Wong, and K. S. Tee. Development of an electronic aerosol system for generating microcapsules. *Journal Teknologi.* 2016. 78(5-7): 79 - 85.

[142] D. Ma, C. Liu, Z. Zhao, and H. Zhang. Rolling friction and energy dissipation in a spinning disc. *Proceedings of the Royal Society A-Mathematical Physical and Engineering Science.* 2014. 470(2169): 20140191.

[143] N. J. Zuidam, and E. Shimoni. Overview of microencapsulates for use in food products or processes and methods to make them. In: *Encapsulation Technologies for Active Foos Ingredients and Food Processing*. Springer: Springer. 2010. 400.

[144] D. Mark, S. Haeberle, R. Zengerle, J. Ducree, and G. T. Vladisavljevic. Manufacture of chitosan microbeads using centrifugally driven flow of gel-forming solutions through a polymeric micronozzle. *Journal of Colloid and Interface Science.* 2009. 336(2): 634 - 641.

[145] W. Krasaekoopt, B. Bhandari, and H. Deeth. Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal.* 2003. 13(1): 3 - 13.

[146] W. Krasaekoopt. Microencapsulation of probiotics in hydrocolloid gel matrices: a review. *Agro Food Industry Hi-Tech.* 2013. 24(2): 76 - 84.

[147] J. Wang, Y. Cheng, Y. Yu, F. Fu, Z. Chen, Y. Zhao, and Z. Gu. Microfluidic generation of porous microcarriers for three-dimensional cell culture. *ACS Applied Materials & Interfaces.* 2015. 7(49): 27035 - 27039.

[148] W. Wang, M. J. Zhang, and L. Y. Chu. Functional polymeric microparticles engineered from controllable microfluidic emulsions. *Accounts of chemical Research.* 2014. 47(2): 373 - 384.

[149] W. L. Chou, P. Y. Lee, C. L. Yang, W. Y. Huang, and Y. S. Lin. Recent advances in applications of droplet microfluidics. *Micromachines.* 2015. 6: 1249 - 1271.

[150] K. Jiang, P. C. Thomas, S. P. Forry, D. L. DeVoe, and S. R. Raghavan. Microfluidic synthesis of monodisperse PDMS microbeads as discrete oxygen sensors. *Soft Matter.* 2012. 8(4): 923 - 926.

[151] K. S. Huang, T. H. Lai, and Y. C. Lin. Manipulating the generation of ca-alginate microspheres using microfluidic channels-as a carrier of gold nanoparticles. Lab On A Chip. 2006. 6(7): 954 - 957.

[152] D. Choi, H. Lee, J. Im do, I. S. Kang, G. Lim, D. S. Kim, and K. H. Kang. Spontaneous electrical charging of droplets by conventional pipetting. *Scientific Reports.* 2013. 3: 2037.

[153] L. Zhang, J. Huang, T. Si, and R. X. Xu. Coaxial electrospray of microparticles and nanoparticles for biomedical applications. *Expert Review of Medical Devices.* 2012. 9(6): 595 - 612.

[154] L. Canaple, A. Rehor, and D. Hunkeler. Improving cell encapsulation through size control. *Journal of Biomaterials Science-Polymer Edition.* 2002. 13(7): 783 - 796.

[155] S. Bigdeli, R. O. Dettloff, C. W. Frank, R. W. Davis, and L. D. Crosby. A simple method for encapsulating single cells in alginate microspheres allows for direct PCR and whole genome amplification. *PLoS One.* 2015. 10(2): e0117738.

[156] P. Kulkarni, P. A. Baron, and K. Willeke. Introduction to aerosol characterization. In: *Aerosol Measurement: Principles, Techniques, and Applications*. New Jersey: John Wiley & Sons, Inc. 2011. 3 - 13.

[157] J. L. Clancy. *Aerodynamics*. India: Sterling Book House. 2006.

[158] G. Najafpour. *Biochemical Engineering and Biotechnology*. 2nd Ed. Elsevier, USA: Elsevier B.V. 2015.

[159] G. J. Mattamal. US FDA perspective on the regulations of medical-grade polymers: cyanoacrylate polymer medical device tissue adhesives. *Expert Review of Medical Devices.* 2008. 5(1): 41 - 49.

[160] A. Batorsky, J. Liao, A. W. Lund, G. E. Plopper, and J. P. Stegemann. Encapsulation of adult human mesenchymal stem cells within collagen-agarose microenvironments. *Biotechnology and Bioengineering.* 2005. 92(4): 492 - 500.

[161] L. Wang, R. R. Rao, and J. P. Stegemann. Delivery of mesenchymal stem cells in chitosan/collagen microbeads for orthopaedic tissue repair. *Cells Tissues Organs.* 2013. 197(5): 333 - 343.

[162] V. X. Truong, K. M. Tsang, G. P. Simon, R. L. Boyd, R. A. Evans, H. Thissen, and J. S. Forsythe. Photodegradable gelatin-based hydrogels prepared by bioorthogonal click chemistry for cell encapsulation and release. *Biomacromolecules.* 2015. 16(7): 2246 - 2253.

[163] B. Sarker, R. Singh, R. Silva, J. A. Roether, J. Kaschta, R. Detsch, D. W. Schubert, I. Cicha, and A. R. Boccaccini. Evaluation of fibroblasts adhesion and proliferation on alginate-gelatin crosslinked hydrogel. *PLoS One.* 2014. 9(9): e107952.

[164] S. N. Tzouanas, A. K. Ekenseair, F. K. Kasper, and A. G. Mikos. Mesenchymal stem cell and gelatin microparticle encapsulation in thermally and chemically gelling injectable hydrogels for tissue engineering. *Journal of Biomedical Materials Research Part A.* 2014. 102(5): 1222 - 1230.

[165] S. Sakai, S. Ito, H. Inagaki, K. Hirose, T. Matsuyama, M. Taya, and K. Kawakami. Cell-enclosing gelatin-based microcapsule production for tissue engineering using a microfluidic flow-focusing system. *Biomicrofluidics*. 2011. 5(013402): 1 - 7.

[166] P. Mercier, F. Fernandez, F. Tortosa, H. Bagheri, H. Duplan, M. Tafani, J. C. Bes, R. Bastide, Y. Lazorthes, and B. Sallerin. A new method for encapsulation of living cells: preliminary results with PC12 cell line. *Journal of Microencapsulation.* 2001. 18(3): 323 - 334.

[167] C. R. Correia, P. Sher, R. L. Reisa, and J. F. Mano. Liquified chitosan–alginate multilayer capsules incorporating poly(L-lactic acid) microparticles as cell carriers. *Soft Matter*. 2013. 9: 2125 - 2130.

[168] S. Ramesh, K. Rajagopal, D. Vaikkath, P. D. Nair, and Madhuri. Enhanced encapsulation of chondrocytes within a chitosan/hyaluronic acid hydrogel: a new technique. *Biotechnology Letters*. 2014. 36(5): 1107 - 1111.

[169] S. Sakai, K. Kawabata, T. Ono, H. Ijima, and K. Kawakami. Development of mammalian cell-enclosing subsieve-size agarose capsules (<100 micron) for cell therapy. *Biomaterials.* 2005. 26(23): 4786 - 4792.

[170] H. Uludag, P. De Vos, and P. A. Tresco. Technology of mamalian cell encapsulation. *Advanced Drug Delivery Reviews.* 2000. 42(1-2): 29 - 64.

[171] P. De Vos, M. M. Faas, B. Strand, and R. Calafiore. Alginate-based microcapsules for immunoisolation of pancreatic islets. *Biomaterials.* 2006. 27(32): 5603 - 5617.

[172] K. A. Heald, T. R. Jay, and R. Downing. Assessment of the reproducibility of alginate encapsulation of pancreatic islets using the MTT colorimetric assay. *Cell Transplantation.* 1994. 3(4): 333 - 337.

[173] L. Picariello, S. Benvenuti, R. Recenti, L. Formigli, A. Falchetti, A. Morelli, L. Masi, F. Tonelli, P. Cicchi, and M. L. Brandi. Microencapsulation of human parathyroid cells: an “in vitro” study. *Journal of Surgical Research*. 2001. 96(1): 81 - 89.

[174] M. H. Wu and W. C. Pan. Development of microfluidic alginate microbead generator tunable by pulsed airflow injection for the microencapsulation of cells. *Microfluidics and Nanofluidics*. 2010. 8(6): 823 - 835.

[175] I. Batubara, D. Rahayu, K. Mohamad, and W. E. Prasetyaningtyas. Leydig cells encapsulation with alginate-chitosan: optimization of microcapsule formation. *Journal of Encapsulation and Adsorption Sciences*. 2012. 2(2): 15 - 20.

[176] T. Moustafa, S. Girod, F. Tortosa, R. Li, J. C. Sol, F. Rodriguez, R. Bastide, Y. Lazorthes, and B. Sallerin. Viability and functionality of bovine chromaffin cells encapsulated into alginate - PLL microcapsules with a liquefied inner core. *Cell Transplantation*. 2006. 15(2): 121 - 133.

[177] T. Andersen, P. Auk-Emblem, and M. Dornish. Review 3D cell culture in alginate hydrogels. *Microarrays*. 2015. 4(2): 133 - 161.

[178] G. M. Lee. and B. O. Palsson. Stability of antibody productivity is improved when hybridoma cells are entrapped in calcium alginate beads. *Biotechnology and Bioengineering*. 1993. 42(9): 1131 - 1135.

[179] M. S. Sinacore, B. C. Creswick, and R. Buehler. Entrapment and growth of murine hybridoma cells in calcium alginate gel microbeads. *Nature Publishing Company*. 1989. 7(12): 1275 - 1279.

[180] S. Overgaard, J. M. Scharer, M. Moo-Young, and N. C. Bols. Immobilization of hybridoma cells in chitosan alginate beads. *The Canadian Journal of Chemical Engineering*. 1991. 69(2): 439 - 443.

[181] M. L. Plunkett and J. A. Hailey. An in vivo quantitative angiogenesis model using tumor cells entrapped in alginate. *Laboratory Investigation*. 1990. 62(4): 510 - 517.

[182] Y. Wang, Q. Chen, and F. Yuan. Alginate encapsulation is a highly reproducible method for tumor cell implantation in dorsal skinfold chambers. *BioTechniques*. 2005. 39(6): 834 - 839.

[183] V. Breguet, R. Gugerli, U. V. Stockar, and I. W. Marison. CHO immobilization in alginate/poly-l-lysine microcapsules: an understanding of potential and limitations. *Cytotechnology*. 2007. 53(1-3): 81 - 93.

[184] J. Xiea and C. H. Wang. Electrospray in the dripping mode for cell microencapsulation. *Journal of Colloid and Interface Science*. 2007. 312(2): 247 - 255.

[185] S. Jitraruch, A. Dhawan, R. D. Hughes, C. Filippi, D. Soong, C. Philippeos, S. C. Lehec, N. D. Heaton, M. S. Longhi, and R. R. Mitry. Alginate microencapsulated hepatocytes optimised for transplantation in acute liver failure. *PLoS One*. 2014. 9(12): 1 - 23.

[186] E. Um, D. S. Lee, H. B. Pyo, and J. K. Park. Continuous generation of hydrogel beads and encapsulation of biological materials using a microfluidic droplet merging channel. *Microfluidics and Nanofluidics*. 2008. 5(4): 541 - 549.

[187] C. Kim, K. S. Lee, Y. E. Kim, K. J. Lee, S. H. Lee, T. S. Kim, and J. Y. Kang. Rapid exchange of oil-phase in microencapsulation chip to enhance cell viability. *Lab On A Chip*. 2009. 9(9): 1294 - 1297.

[188] R. Rathinamoorthy and L. Sasikala. Polysaccharide fibers in wound management. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2011. 3(3): 38 - 44.

[189] V. V. Malkar, T. Mukherjee, and S. Kapoor. Aminopolycarboxylic acids and alginate composite-mediated green synthesis of Au and Ag nanoparticles. *Journal of Nanostructure in Chemistry.* 2015. 5(1): 1 - 6.

[190] J. E. Wintter, W. M. Lauter, and P. A. Foote. Derivatives of alginic acid. *Journal of the American Pharmaceutical Association.* 1955. 44(1): 48 - 51.

[191] M. Janarthanan and K. M. Buvaeswari. Seaweeds used as a source of alginate in textile industry. *International Journal of Current Research and Development.* 2014. 2(1): 48 - 55.

[192] L. Pereira, S. F. Gheda, and P. J. A. Ribeiro-Claro. Analysis by vibrational spectroscopy of seaweed polysaccharides with potential use in food, pharmaceutical, and cosmetic industries. *International Journal of Carbohydrate Chemistry.* 2013. 2013(537202): 1 - 7.

[193] S. Pistone, D. Qoragllu, G. Smistad, and M. Hiorth. Formulation and preparation of stable cross-linked alginate-zinc nanoparticles in the presence of a monovalent salt. *Soft Matter.* 2015. 11(28): 5765 - 5774.

[194] T. Sone, E. Nagamori, T. Ikeuchi, A. Mizukami, Y. Takakura, S. Kajiyama, E. Fukusaki, S. Harashima, A. Kobayashi, and K. Fukui. A novel gene delivery system in plants with calcium alginate micro-beads. *Journal of Bioscience and Bioengineering.* 2002. 94(1): 87 - 91.

[195] Y. Zhou, S. Kajiyama, H. Masuhara, Y. Hosokawa, T. Kaji, and K. Fukui. A new size and shape controlling method for producing calcium alginate beads with

immobilized proteins. *Journal of Biomedical Science and Engineering*. 2009. 2(5): 287 - 293.

[196] O. Smidsrod and G. Skjak-Brik. Alginate as immobilization matrix for cells. *Trends in Biotechnology*. 1990. 8: 71 - 78.

[197] W. K. Majure, N. W. Vandervort, P. J. David, B. D. Deeter, G. J. Melchior, J. R. Brazzle, K. R. Muzyk, F. R. Miranda, T. W. Cheung, and O. G. Bain. *Plant treatment compositions and methods for their use*. Patent EP2608674A1. 2012.

[198] D. Murphy. *Phase Contrast Microscopy In Fundamentals of Light Microscopy and Digital Imaging*. New York: Wiley-Liss. 2001.

[199] M. Hoppert. *Microscopic Techniques in Biotechnology*. Germany: Wiley-Vch Verlag. 2003.

[200] K. Maxwell and G. N. Johnson. Chlorophyll fluorescence-a practical guide. *Journal of Experimental Botany.* 2000. 51(345): 659 - 668.

[201] H. C. Ishikawa-Ankerhold, R. Ankerhold, and G. P. Drummen. Advanced fluorescence microscopy techniques--FRAP, FLIP, FLAP, FRET and FLIM. *Molecules.* 2012. 17(4): 4047 - 4132.

[202] M. Havrdova, K. Polakova, J. Skopalik, M. Vujtek, A. Mokdad, M. Homolkova, J. Tucek, J. Nebesarova, and R. Zboril. Field emission scanning electron microscopy (FE-SEM) as an approach for nanoparticle detection inside cells. *Micron.* 2014. 67: 149 - 154.

[203] Y. Yuan, Y. Shimada, S. Ichinose, and J. Tagami. Qualitative analysis of adhesive interface nanoleakage using FE-SEM/EDS. *Dental Materials.* 2007. 23(5): 561 - 569.

[204] Y. J. Kim and C. R. Park. Technical review: principle of field emission-scanning electron microscopy (FE-SEM) and its application to the analysis of carbon nanostructures. *Carbon Letters*. 2001. 2(3&4): 202 - 211.

[205] R. J. Markovich and C. Pidgeon. Introduction to fourier transform infrared spectroscopy and applications in the pharmaceutical sciences. *Pharmaceutical Research.* 1991. 8(6): 663 - 675.

[206] A. A. Baravkar, R. N. Kale, and S. D. Sawant. FTIR Spectroscopy: principle, technique and mathematics. *International Journal of Pharma and Bio Sciences.* 2011. 2(1): 513 - 519.

[207] J. Kapuscinski. DAPI: a DNA-specific fluorescent probe. *Biotechnic & Histochemistry*. 1995. 70(5): 220 - 233.

[208] G. Manzini, M. L. Barcellona, M. Avitabile, and F. Quadrifoglio. Interaction of diamidino-2-phenylindole (DAPI) with natural and synthetic nucleic acids. *Nucleic Acids Research.* 1983. 11(24): 8861 - 8876.

[209] A. Sukenik, O. Hadas, S. Stojkovic, N. Malinsky-Rushansky, Y. Viner-Motzini, and J. Beardall. Fluorescence microscopy reveals variations in cellular composition during formation of akinetes in the cyanobacterium Aphanizomenon ovalisporum. *European Journal of Phycology.* 2009. 43(3): 309 - 317.

[210] N. Spackova, T. E. Cheatham, F. Ryjacek, F. Lankas, L. Van Meervelt, P. Hobza, and J. Sponer. Molecular dynamics simulations and thermodynamics analysis of DNA-drug complexes. Minor groove binding between 4',6-diamidino-2-phenylindole and DNA duplexes in solution. *Journal of the American Chemical Society.* 2003. 125(7): 1759 - 1769.

[211] D. E. Pegg. Viability assays for preserved cells, tissues, and organs. *Cryobiology.* 1989. 26(3): 212 - 231.

[212] A. W. Hayes and R. Press. *Principles and Methods of Toxicology*. New York: CRC Press Taylor & Francis. 1994.

[213] V. Breguet, U. V. Stockar, and I. W. Marison. Characterization of alginate lyase activity on liquid, gelled, and complexed states of alginate. *Biotechnology Progress.* 2007. 23(5): 1223 - 1230.

[214] R. J. Trotman, C. E. Camp, A. Ben-Bassat, R. DiCosimo, L. Huang, G. A. Crum, F. S. Sariaslani, and S. L. Haynie. Calcium alginate bead immobilization of cells containing tyrosine ammonia lyase activity for use in the production of p-hydroxycinnamic acid. *Biotechnology Progress.* 2007. 23(3): 638 - 644.

[215] Y. Oh, X. Xu, J. Y. Kim, and J. M. Park. Maximizing the utilization of Laminaria japonica as biomass via improvement of alginate lyase activity in a

two-phase fermentation system. *Biotechnology Journal.* 2015. 10(8): 1281 - 1288.

[216] J. B. Hansen and L. K. Nakamura. Distribution of alginate lyase activity among strains of bacillus circulans. *Applied and Environmental Microbiology.* 1985. 49(4): 1019 - 1021.

[217] S. Sakai, S. Ito, Y. Ogushi, I. Hashimoto, N. Hosoda, Y. Sawae, and K. Kawakami. Enzymatically fabricated and degradable microcapsules for production of multicellular spheroids with well-defined diameters of less than 150 microm. *Biomaterials.* 2009. 30(30): 5937 - 5942.

[218] B. Zhu and H. Yin. Alginate lyase: review of major sources and classification, properties, structure-function analysis and applications. *Bioengineered.* 2015. 6(3): 125 - 131.

[219] T. Y. Wong, L. A. Preston, and N. L. Schiller. Alginate lyase: review of major sources and enzyme characteristics, structure-function analysis, biological roles, and applications. *Annual Review of Microbiology.* 2000. 54: 289 - 340.

[220] H. S. Kim, C.-G. Lee, and E. Y. Lee. Alginate lyase: structure, property, and application. *Biotechnology and Bioprocess Engineering.* 2011. 16(5): 843 - 851.

[221] P. Soni and K. Suchdeo. Exploring the serial capabilities for 16x2 LCD interface. *International Journal of Emerging Technology and Advanced Engineering.* 2012. 2(11): 109 - 112.

[222] I. Notingher, J. R. Jones, S. Verrier, I. Bisson, P. Embanga, P. Edwards, J. M. Polak, and L. L. Hench. Application of FTIR and Raman spectroscopy to characterisation of bioactive materials and living cells. *Spectroscopy.* 2003. 17(2, 3): 275 - 288.

[223] K. T. Thong. *Development of a novel liquid crystal based 3D micro-tissues culture technique and microfluidic vibrational cleaner*. Master Thesis. Universiti Tun Hussein Onn Malaysia. 2016.

[224] J. T. Whitton and J. D. Everall. The thickness of the epidermis. *The British Journal of Dermatology.* 1973. 89(5): 467 - 476.

[225] P. Smrdel, M. Bogataj, and A. Mrhar. The influence of selected parameters on the size and shape of alginate beads prepared by ionotropic gelation. *Journal of Pharmaceutical Sciences*. 2008. 76(1): 77 - 89.

[226] D. F. Elger, B. C. Williams, C. T. Crowe, and J. A. Roberson. *Engineering Fluid Mechanics*. 10th Ed. Singapore: John Wiley & Sons Singapore. 2014.

[227] J. Zhao and L. Yang. Simulation and experimental study on the atomization character of the pressure-swirl nozzle. *Open Journal of Fluid Dynamics.* 2012. 2(4A): 271 - 277.

[228] H. S. M. Aly, M. N. M. Jaafar, and T. M. Lazim. *Spray and atomization characterictics of liquid*. 1st Ed. Malaysia: Penerbit UTM Press. 2013.

[229] B. Watson. *Mobile Equipment Hydraulics: A Systems and Troubleshooting Approach*. USA: Delmar Cengage Learning. 2010.

[230] A. Surjosatyo and F. N. Ani. A numerical study of air flow in a coaxial pipe. *Jurnal Teknologi.* 2001. 24(A): 1 - 15.

[231] S. Haeberle, L. Naegele, R. Burger, F. Von Stetten, R. Zengerle, and J. Ducree. Alginate bead fabrication and encapsulation of living cells under centrifugally induced artificial gravity conditions. *Journal of Microencapsulation.* 2008. 25(4): 267 - 274.

[232] D. Gareau. Automated identification of epidermal keratinocytes in reflectance confocal microscopy. *Journal of Biomedical Optics.* 2011. 16(3): 030502.

[233] R. Sharma. Gadolinium toxicity: epidermis thickness measurement by magnetic resonance imaging at 500 MHz. *Skin Research and Technology.* 2010. 16(3): 339 - 353.

[234] S. B. Hoath and D. G. Leahy. The organization of human epidermis: functional epidermal units and phi proportionality. *The Journal of Investigative Dermatology.* 2003. 121(6): 1440 - 1446.

[235] F. A. Leblond, G. Simard, N. Henley, B. Rocheleau, P. M. Huet, and J. P. Halle. Studies on smaller (approximately 315 micron) microcapsules: IV. Feasibility and safety of intrahepatic implantations of small alginate poly-L-lysine microcapsules. *Cell Transplantation.* 1999. 8(3): 327 - 337.

[236] P. Lee and M. A. Rogersn. Effect of calcium source and exposure-time on basic caviar spherification using sodium alginate. *International Journal of Gastronomy and Food Science.* 2012. 1(2): 96 - 100.

[237] H. J. Kong, D. Kaigler, K. Kim, and D. J. Mooney. Controlling rigidity and degradation of alginate hydrogels via molecular weight distribution. *Biomacromolecules.* 2004. 5(5): 1720 - 1727.

[238] M. Guvendiren and J. A. Burdick. Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. *Nature Communications.* 2012. 3: 792.

[239] N. Cao, X. B. Chen, and D. J. Schreyer. Influence of calcium ions on cell survival and proliferation in the context of an alginate hydrogel. *ISRN Chemical Engineering.* 2012. 2012(516461): 9.

[240] W. Zhang and X. He. Microencapsulating and banking living cells for cell-based medicine. *Journal of Healthcare Engineering.* 2011. 2(4): 427 - 446.

[241] J. Friedrich, R. Ebner, and L. A. Kunz-Schughart. Experimental anti-tumor therapy in 3-D: spheroids-old hat or new challenge. *International Journal of Radiation Biology.* 2007. 83(11-12): 849 - 871.

[242] C. Y. Chen, C. J. Ke, K. C. Yen, H. C. Hsieh, J. S. Sun, and F. H. Lin. 3D porous calcium-alginate scaffolds cell culture system improved human osteoblast cell clusters for cell therapy. *Theranostics.* 2015. 5(6): 643 - 655.

[243] S. V. Bhujbal, B. de Haan, S. P. Niclou, and P. de Vos. A novel multilayer immunoisolating encapsulation system overcoming protrusion of cells. *Scientific Reports.* 2014. 4: 6856.

[244] K. Kevekordes. Using light scattering measurements to study the effects of monovalent and divalent cations on alginate aggregates. *Journal Of Experimental Botany.* 1996. 47(298): 677 - 682.

[245] M. Nagpal, S. K. Singh, and D. Mishra. Synthesis characterization and in vitro drug release from acrylamide and sodium alginate based superporous hydrogel devices. *International Journal of Pharmaceutical Investigation.* 2013. 3(3): 131 - 140.

[246] H. Daemi and M. Barikani. Synthesis and characterization of calcium alginate nanoparticles, sodium homopolymannuronate salt and its calcium nanoparticles. *Scientia Iranica*. 2012. 19(6): 2023 - 2028.

[247] D. Dianawati, V. Mishra, and N. P. Shah. Role of calcium alginate and mannitol in protecting Bifidobacterium. *Applied and Environmental Microbiology.* 2012. 78(19): 6914 - 6921.

[248] K. Kesavan, G. Nath, and J. K. Pandit. Sodium alginate based mucoadhesive system for gatifloxacin and its in vitro antibacterial activity. *Scientia Pharmaceutica.* 2010. 78(4): 941 - 957.

[249] Z. Movasaghi, S. Rehman, and I. U. Rehman. Fourier transform infrared (FTIR) spectroscopy of biological tissues. *Applied Spectroscopy Reviews.* 2008. 43: 134 - 179.

[250] E. P. U. Otero, G. S. N. Eliel, E. J. S. Fonseca, J. M. Hickmann, R. Rodarte, E. Barretoc, and K. J. Jalkanen. Study of cancer cell lines with Fourier transform infrared (FTIR)/vibrational absorption (VA) spectroscopy. *Current Physical Chemistry.* 2012. 2: 1 - 14.

[251] P. Smrdel, M. Bogataj, and A. Mrhar. The influence of selected parameters on the size and shape of alginate beads prepared by ionotropic gelation. *Journal of Pharmaceutical Sciences*. 2008. 76(1): 77 - 89.

[252] Q. Zuo, J. Lu, A. Hong, D. Zhong, S. Xie, Q. Liu, Y. Huang, Y. Shi, L. He, and W. Xue. Preparation and characterization of PEM-coated alginate microgels for controlled release of protein. *Biomedical Materials.* 2012. 7(3): 035012.

[253] K. Horigome, T. Ueki, and D. Suzuki. Direct visualization of swollen microgels by scanning electron microscopy using ionic liquids. *Polymer Journal.* 2016. 48(3): 273 - 279.

[254] Y. R. Lou, L. Kanninen, B. Kaehr, J. L. Townson, J. Niklander, R. Harjumaki, C. Jeffrey Brinker, and M. Yliperttula. Silica bioreplication preserves three-dimensional spheroid structures of human pluripotent stem cells and HepG2 cells. *Scientific Reports.* 2015. 5: 13635.

[255] J. Paul. The cancer cell in vitro: a review. *Cancer Research.* 1962. 22: 431 - 440.

[256] R. J. DeBerardinis, J. J. Lum, G. Hatzivassiliou, and C. B. Thompson. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metabolism.* 2008. 7(1): 11 - 20.

[257] R. A. Cairns, I. S. Harris, and T. W. Mak. Regulation of cancer cell metabolism. *Nature Reviews Cancer.* 2011. 11(2): 85 - 95.

[258] D. B. Nandini and R. V. Subramanyam. Nuclear features in oral squamous cell carcinoma: a computer-assisted microscopic study. *Journal of Oral and Maxillofacial Pathology.* 2011. 15(2): 177 - 181.

[259] D. Zink, A. H. Fischer, and J. A. Nickerson. Nuclear structure in cancer cells. *Nature Reviews Cancer.* 2004. 4(9): 677 - 687.

[260] C. K. Griffith, C. Miller, R. C. Sainson, J. W. Calvert, N. L. Jeon, C. C. Hughes, and S. C. George. Diffusion limits of an in vitro thick prevascularized tissue. *Tissue Engineering.* 2005. 11(1-2): 257 - 266.

[261] S. Sakai, I. Hashimoto, Y. Ogushi, and K. Kawakami. Peroxidase-catalyzed cell encapsulation in subsieve-size capsules of alginate with phenol moieties in water-immiscible fluid dissolving H_2O_2. *Biomacromolecules.* 2007. 8(8): 2622 - 2626.

[262] C. F. Amstein and P. A. Hartman. Adaptation of plastic surfaces for tissue culture by glow discharge. *Journal of Clinical Microbiology.* 1975. 2(1): 46 - 54.

[263] P. Thevenot, W. Hu, and L. Tang. Surface chemistry influence implant biocompatibility. *Current Topics in Medicinal Chemistry.* 2008. 8(4): 270 - 280.

[264] A. S. Curtis, J. V. Forrester, C. McInnes, and F. Lawrie. Adhesion of cells to polystyrene surfaces. *The Journal of Cell Biology.* 1983. 97(5 Pt 1): 1500 - 1506.

[265] W. S. Ramsey, W. Hertl, E. D. Nowlan, and N. J. Binkowski. Surface treatments and cell attachment. *In Vitro Cellular & Developmental Biology-Animal.* 1984. 20(10): 802 - 808.