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DEVELOPMENT OF A FLICKING SYSTEM FOR PRODUCING CALCIUM ALGINATE MICROBEADS

Soon Chuan Wong[a], Chin Fhong Soon[b*], Wai Yean Leong[a], Kian Sek Tee[a]

[a]Faculty of Electrical and Electronic Engineering, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia
[b]Biosensor and Bioengineering Laboratory, MiNT-SRC Research Center, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Batu Pahat, Johor, Malaysia

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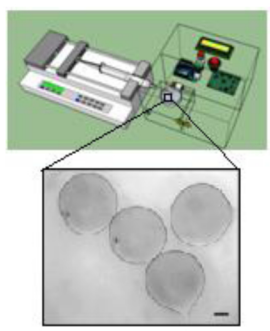
*Corresponding author
soon@uthm.edu.my

Graphical abstract



Abstract

Microencapsulation of cells for various applications is attracting increasing interest. A variety of microencapsulation technology has been developed to produce cells encapsulation. However, previous microencapsulation systems were associated with complex design, high voltage supply, requirements of post cleaning process and large volume of reagents. In this paper, we proposed the development of a flicking device for generating a 3D cell model in calcium alginate microbeads that has potential for pharmacological test and tissue implants. A flicking system based on a flicking device and a syringe pump has been developed for generation of calcium alginate microbeads. The size of the microbeads produced using this system can be controlled by changing the flow rate (5 to 15 µl/min) of the syringe pump and fixing the motor rotation speed of the flicking device at 90 rpm. The calcium alginate microbeads produced using the flicking device were shown to be size controllable (270 to 430 µm) and suitable for microencapsulation.

Keywords: Flicking; calcium alginate; microbeads; pulse width modulation; motor

1.0 INTRODUCTION

Microcapsule is a sphere with the size varying between 50 nm to 2 mm and the core is used to contain substance [1]. Microcapsules have wide applications in drug release, tissue engineering and food science [1]. There are various types of materials that can be used to produce the microcapsules such as agarose, collagen, alginate, chitosan and gelatin [2]. Alginate can be physically crosslinked to form a gel phase with divalent ions [3]. Of all the synthetic and natural sources of hydrogel materials, alginate is attractive for encapsulating living cells. The cells encapsulated in microcapsules are useful as biomaterials or fillers to the artificial tissues [4, 5]. Cell encapsulation technology in alginate, which is a natural biodegradable polymer that mimics the extracellular matrix and supports both cell functions and metabolism, has been developed with an aim to obtain three-dimensional (3D) cultures [5]. In tissue engineering, microencapsulation of cells in sodium alginate were potentially applied in immunoisolatory and biochemical assays [6]. The microencapsulated cells can be transplanted because the micropasules are biocompatible for both the host and enclosed cells [5].

Encapsulation technology must fulfill the strict requirements that are applicable to therapeutic strategies. Various considerations in terms of performance, biosafety, biocompatibility, retrievability, stability, availability, purity, characterisation and cost are of paramount importance [7]. A few methods that have been developed for microencapsulation of cells were based on microfluidic device [8], emulsification [9], extrusion [10], electrospray [11], electrostatic [12] and airflow [13]. However, previous methods reported were presented with a few disadvantages such as complex design, high voltage supply, requirement of post cleaning process and large

volume of reagents. Calcium alginate beads can be easily fabricated by extruding drops of sodium alginate solution in air using a syringe and collecting these drops into calcium chloride solution, it offers little control of drop formation and millimeter-sized beads. Micrometer-sized beads provide higher mechanical strength, easier implantation and better transport of oxygen and nutrients [14]. Although alginate hydrogel beads can be fabricated by using microfluidic approach in micron size, the alginate microbeads formed were covered with oil layer, post cleaning process is required to remove the oil layer before dealing with cells. The removal of the immiscible fluid requires strong mechanical and chemical treatments, which prolongs exposure of the cells to divalent ions or solvents [8]. Alterantively, microbeads generation techniques assisted by airflow, electrostatic field and electrospray have decrease the alginate drop size and made its distribution relatively narrow. However, it required large volume of reagents in alginate beads fabrication and sophisticated high voltage supply to create atomization of alginate droplets. The purpose of this study is to develop a flicking device that could be used to produce calcium alginate (CaAlg) microbeads for the encapsulation of cells. The proposed method is simple, economic and reagent saving.

2.0 MATERIALS AND METHODS

2.1 Development of A Flicking Device

Development of the flicking device is according to the flow chart as shown in Figure 1. Firstly, the conceptual model of the prototype for the flicking device was designed by using a Sketchup Pro 2014 software. Then, the circuits of the flicking device were tested on a breadboard before further developed into a printed circuit board (PCB). Next, the performance of the designed circuit was tested with various flow rates (µl / min) and flicking speed (revolutions per minute). Images of the microcapsules from the flicker device was captured using a Nikon (Eclipse TS100, Japan) inverted phase contrast microscope, fixed with a QImaging (Go-3, Canada) charge-coupled device (CCD) camera. Based on the images captured, the size of the microbeads generated by different flow rates and motor speeds were measured and characterised.

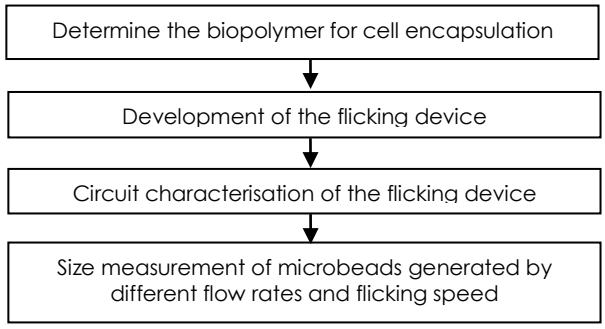


Figure 1 Flow chart for the development of a flicking device

The components used in the development of flicking device were a lithium ion rechargeable battery (+12 Vdc, 2200 mAh), linear voltage regulator (LM317), Arduino-uno microcontroller, mini DC geared motor (100 rpm, 6.5 kg-cm, 6 V), potentiometer (10k Ω) and a 16 × 2 liquid crystal display (LCD). Figure 2 shows the overall block diagram of the circuits used for the development of a flicking device.A direct current (dc) 12 volt lithium-ion rechargeable battery was used to power the flicking device which allows the device to be portable without using a power adaptor.

A voltage regulator was used to convert the power source of the battery from 12 Vdc into 9 Vdc and then supplied to a DC geared motor and Arduino-uno microcontroller. Then, the microcontroller was used to generate a PWM signal to control the motor speed and read the current voltage level of the battery. The mini DC geared motor was specified to rotate at 100 rpm and hence, produced 6.5 kg-cm torque when 6 V was applied according to datasheet. At a higher voltage of 9 Vdc, the DC motor was specified to rotate at 150 rpm. Next, potentiometer was used to control the input voltage for microcontroller to generate different duty cycle of PWM signal.

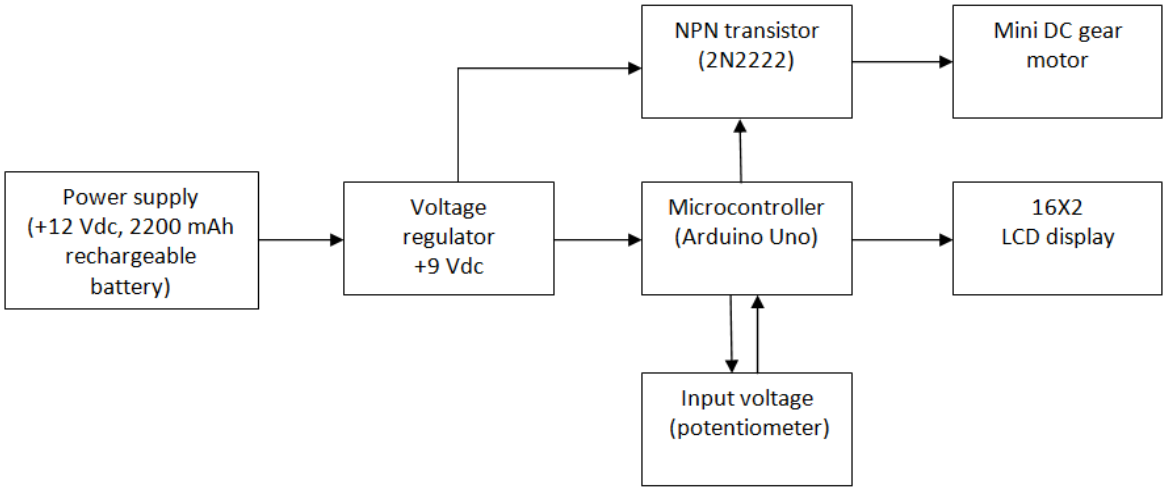


Figure 2 The block diagram of the circuits for the development of a flicking device

When the device was powered, the selected motor speed and battery level indicator would be displayed on a LCD screen as shown in Figure 3. The flicking device was incorporated with a conventional syringe pump to generate CaAlg microbeads. The effects of the motor speed and flow rate of the syringe pump to the size of the microbeads would be discussed.

Figure 3 A LCD display of the flicking device indicating the duty cycle of the PWM and speed of the flicking device

2.2 Programming Workflow of The Flicking Device

The programming flow chart is as shown in Figure 4. The program of the Arduino-uno microcontroller was written in C language and it is function to read the user input from the switches and display the user selection to a LCD. In addition, the program was also designed to read the current voltage level of the battery and user selected motor speed via the voltage of the potentiometer. Next, the microcontroller was programmed to check and read the status of start switch in order to onset the mini geared motor. After the start button was being activated by the user, the motor started to rotate according to the selected motor speed. Otherwise, the motor would not rotate if the start button was not being pressed. Finally, the LCD displays the current speed of motor, battery indication level as well as the duty cycle of the PWM as shown in Figure 3. The PWM output of the circuit designed was captured using an Agilent oscilloscope (DSO-X 2022A) and a digital tachometer (Check line, model CDT-2000HD) was used to measure the rotation speed of the flicking motor.

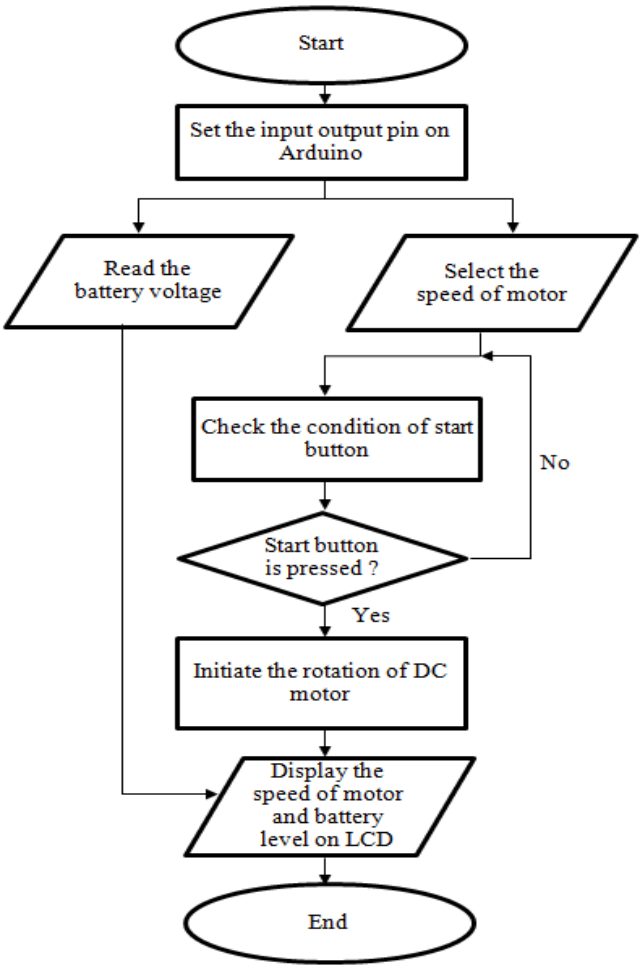


Figure 4 The flow chart for programming the microcontroller

2.3 Preparation of The Alginate Solution and Production of Microbeads

Sodium alginate (W201502-1KG) and calcium chloride (C1016-500G) used in the experiment were purchased from Sigma-Aldrich, United Kingdom. For the preparation of the solutions, calcium chloride and sodium alginate were each mixed separately in the distilled water and prepared in 2 % w/v and 5 % w/v, respectively. They required for the gelation of calcium alginate microbeads later. Then, the solutions were filtered using 0.2 µm PTFE membrane Acrodisc® syringe filter (Pall® Life Sciences). Subsequently, 2 % w/v concentration of sodium alginate solution and 5 % w/v concentration of calcium chloride solution were prepared. A customised system consisting of a flicking device and incorporated with a commercial syringe pump (New Era NE-4002X) was used to generate the CaAlg microbeads (Figure 5). A 0.5 ml insulin syringe purchased from BD Biosciences (Becton, Dickinson and Company, United States) was filled with the sodium alginate solution. The syringe was fixed with a needle of 29 gauge. An ethylene tetrafluoroethylene (ETFE) plastic tie (or cable tie) was used as a flicking flap which was fixed to the shaft of a mini DC geared motor. The rotation of the flicking flap creates a tapping force to the needle of the syringe while the syringe is simultaneously dispensing droplets

of sodium alginate via the needle. The tapping force induced the dispersion of the alginate droplets that subsequently drop into a petri dish containing 4 ml of calcium chloride solution. The petri dish was placed 5 cm directly under the needle of the syringe. Then, the microbeads of the calcium alginate formed were left in the solution for 10 minutes for further gelation. The experiment was repeated 3 times for each flow rate at 5 µl/min, 10 µl/min and 15 µl/min, respectively. A total number of 30 calcium alginate microbeads samples were collected and measured each time for each flow rate. All experiments were performed at room temperature 25 °C for 20 seconds time to produce 30 microbeads.

3.0 RESULTS AND DISCUSSION

3.1 Verification of PWM Signals

Figure 6 (a-e) shows the signal pattern of PWM at 0 %, 25%, 50%, 75% and 100% duty cycle respectively. The period of each complete cycle of a PWM waveform generated by the circuit is 2 ms. 5 volt peak to peak voltage and a frequency of 500 Hz. The PWM signal was used to control motor rotation speed of the flicking device. This result indicates that the designed circuit could generate different PWM signal effectively according to the program in the microcontroller.

3.2 Effect of The Potentiometer Input Voltage to PWM Signal

The potentiometer produced variable voltages ranging from 0 to 5 V to control the PWM signal varying between 0 to 100 % duty cycle. In turn, the PWM signals regulated the motor speed. As shown in Figure 7, the duty cycle of the PWM is linearly proportional to the input voltage of the potentiometer.

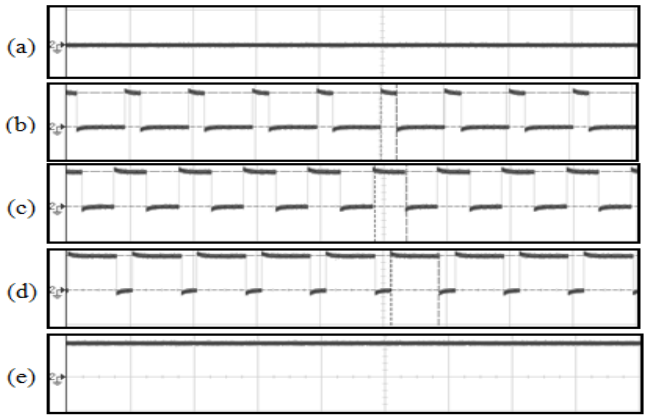


Figure 6 The output signal of PWM shown by oscilloscope: (a) 0 %, (b) 25 %, (c) 50 %, (d) 75 % and (e) 100 % duty cycle

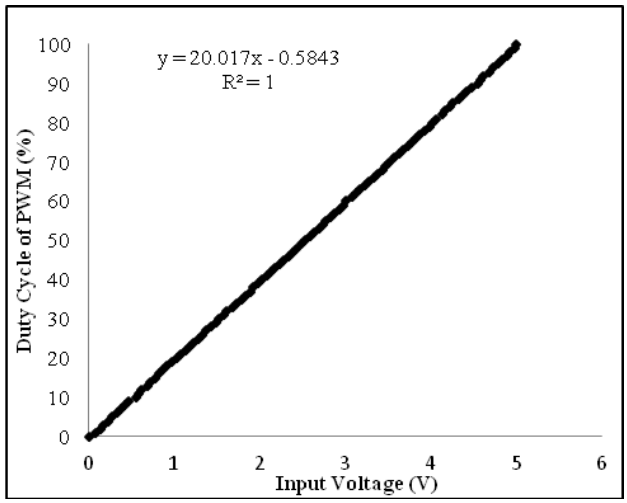


Figure 7 The relationship between the duty cycle of the PWM signal and input voltage of the potentiometer

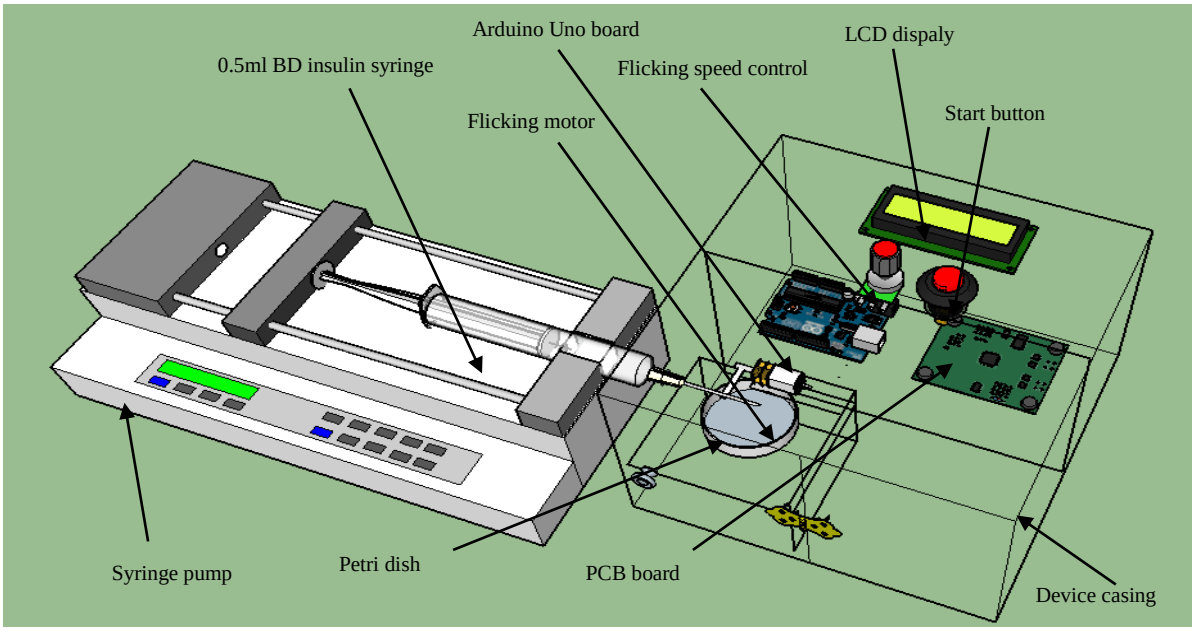


Figure 5 The experimental setup to generate calcium alginate microbead

3.3 Effect of PWM on Motor Speed

The relationship of the duty cycle of PWM and mini DC geared motor speed is as shown in Figure 8. The motor speeds increased gradually with respect to the PWM signals. The mini DC gear motor could not rotate when the PWM signal was lower than 10 % duty cycle. The minimum and maximum rotation speed of the motor was determined at 60 and 150 rpm, respectively (Figure 8). There was a saturation of motor speed at 83 % duty cycle of PWM. Flicking speed is adjustable ranging between 60 and 150 flicking per minute.

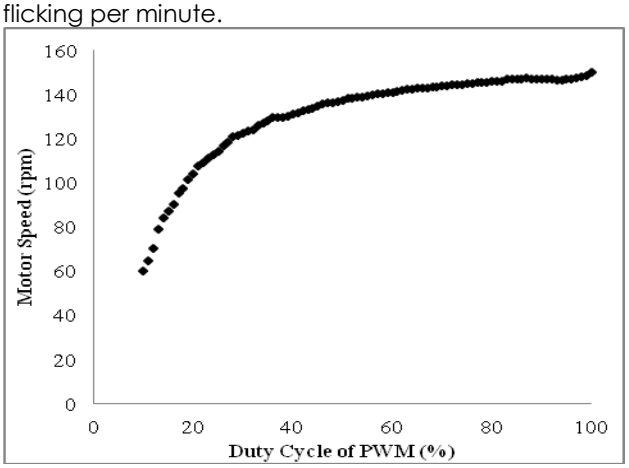


Figure 8 The graph of motor speed versus duty cycle of PWM

3.4 Battery Level Indicator of The Flicking Device

Due to the reason that the flicking device was designed to be portable powered by a rechargeable battery, hence, a battery level indicator is essential to be included in the design. A graphical indicator of battery level was included in the LCD screen which enables the user to know the right time to recharge the battery of the device. The battery level indicator showed a full bar of battery as shown in Figure 9(a) when the battery level was fully charged and the device could perform in an optimum condition. The battery level indicator shows an empty bar when the battery level is on the middle level (Figure 9b). Then, the battery level indicator shows two empty bars when the battery level is low (Figure 9c). When the battery level is in an extremely low level, all bars are empty (Figure 9d).

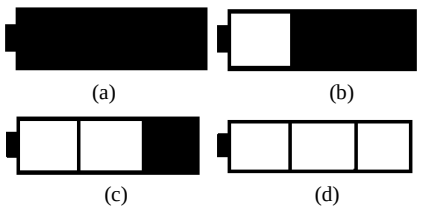


Figure 9 Battery level indicator: (a) high battery level, (b) middle battery level, (c) low battery level and (d) extremely low battery level

3.5 Effects of Flow Rate to The Microbeads Size

At a fixed motor speed of 90 rpm (production rate of 15 microbeads per 10 seconds), variable flow rates (5 µl/min, 10 µl/min, 15 µl/min) of the syringe pump were found to influence the size of the calcium alginate microbeads. The average sizes of calcium alginate microbeads generated by the flow rates of 5 µl/min, 10 µl/min and 15 µl/min were 270.77 ± 22.96 µm, 369.88 ± 4.47 µm and 429.85 ± 40.89 µm, respectively. The size of the alginate beads increased linearly with the flow rate of the syringe pump (Figure 10). Figure 11 shows the different effects of the flow rate to the size of the microbeads.

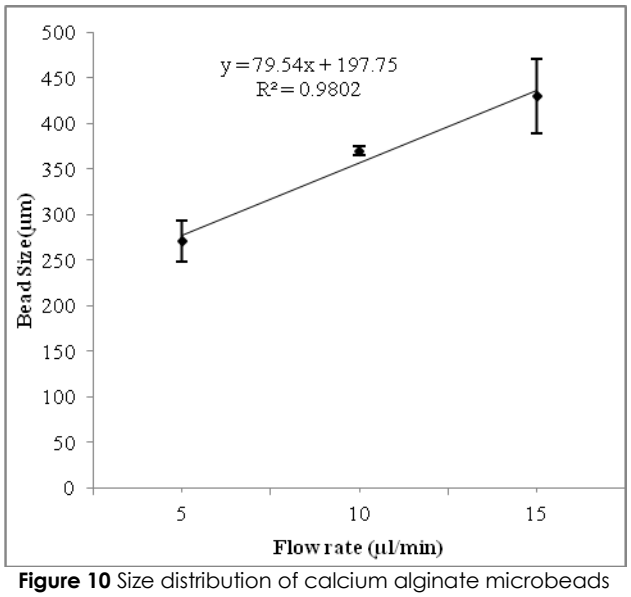


Figure 10 Size distribution of calcium alginate microbeads

From the results, it clearly showed that the size of microbeads generated by flicking method was greatly dependent on the flow rate and speed of the flicking motor. The proper choice of these two parameters can produce a desirable size of microbeads. Smaller microcapsules (less than 300 µm) offer many advantages for the use of cell transplantation as suggested in previous literature [13]. Furthermore, transportation of nutrients and oxygen is theoretically better in smaller microcapsules [15]. The microbeads produced by the flicking method were not presented with several problems as reported previously such as shape deformation [16], tail shape microbeads [17], an undesirable oil layer covering the beads which is difficult to apply to the culturing of living cells [8]. This technique has good potential for the encapsulation of cells in well controlled production of microbeads at room temperature.

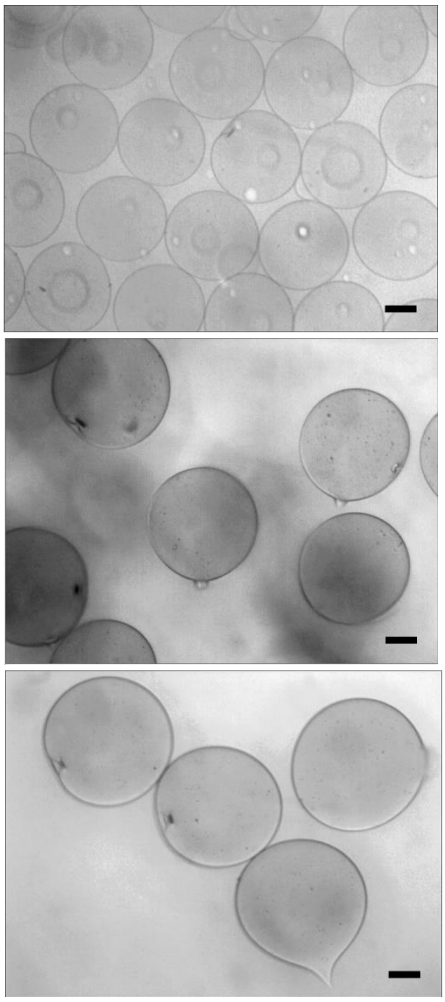


Figure 11 Microbeads samples generated by different flow rates: (a) 5 µl/min, (b) 10 µl/min and (c) 15 µl/min. (Scale bar = 100 µm)

4.0 CONCLUSIONS

A flicking device has been developed to synthesise and fabricate the calcium alginate microbeads with a diameter ranging from 270 to 430 µm under flicking rate of 90 rpm and flow rate of alginate ranging from 5 to 15 µl/min. This was achieved by careful control of the flicking speed of the flicking device and flow rate of the syringe pump. At a fixed flicking speed, the flow rate of the syringe pump directly affects the size of the microbeads generated. Larger size of microbeads was produced with higher flow rate of syringe pump. The flicking method proposed is a very straight forward and simple method to generate calcium alginate microbeads without complex preparation conditions.

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References

[1] Singh M. N., Hemant K. S., Ram M., and Shivakumar H. G.. 2010. Microencapsulation: A Promising Technique For Controlled Drug Delivery. *Res Pharm Sci.* 5(2): 65-77.
[2] Kang, A. Park, J. Ju,J. Jeong,G. S. and Lee S. H.. 2014. Cell Encapsulation Via Microtechnologies. *Biomaterials.* 35(9): 2651-63.
[3] Lee K. Y. and Mooney D. J.. 2001. Hydrogels For Tissue Engineering. *Chem Rev.* 101(7): 1869-79.
[4] Jinchen Sun H. T. 2013. Review Alginate-Based Biomaterials for Regenerative Medicine Applications. *Materials.* 6(4): 1285-1309.
[5] Ghidoni,I. Chlapanidas, T. Bucco,M. Crovato,F. Marazzi, Vigo M. D., *et al.* 2008. Alginate Cell Encapsulation: New Advances In Reproduction And Cartilage Regenerative Medicine. *Cytotechnology.* 58(1): 49-56.
[6] Wang,L. Shelton,R. M. Cooper, P. R. Lawson,M. Triffitt,J. T. and Barralet J. E.. 2003. Evaluation Of Sodium Alginate For Bone Marrow Cell Tissue Engineering. *Biomaterials.* 24(20): 3475-81.
[7] Orive,G. Hernandez,R. M. Rodriguez Gascon,A. Calafiore,R. Chang,T. M. Vos, P. De *Et Al.* 2004. History, Challenges And Perspectives Of Cell Microencapsulation. *Trends Biotechnol.* 22(2): 87-92.
[8] Martinez,C. J. Kim, J. W. Ye,C Ortiz,. I. A. Rowat,C. Marquez,M. *et al.* 2012. A Microfluidic Approach To Encapsulate Living Cells In Uniform Alginate Hydrogel Microparticles. *Macromol Biosci.* 12(7): 946-51.
[9] Raymond,M. C. Neufeld,R. J. and Poncelet.D. 2004. Encapsulation Of Brewers Yeast In Chitosan Coated Carrageenan Microspheres By Emulsification/Thermal Gelation. *Artif Cells Blood Substit Immobil Biotechnol.* 32(2): 275-91.
[10] Rama Dubey,T. C. S. Bhasker Rao.K.U. January 2009. Microencapsulation Technology and Applications. *Defence Science Journal.* 59(1): 82-95.
[11] Xie J. and Wang. C. H. 2007. Electrospray In The Dripping Mode For Cell Microencapsulation. *J Colloid Interface Sci.* 312(2): 247-55.
[12] Dorota Lewinska,J. Marek Kozuchowski,B. Andrzej Kinasiewicz, Andrzej Werynski. 2008. Electrostatic Microencapsulation of Living Cells. *Biocybernetics and Biomedical Engineering.* 28(2): 69–84.
[13] Sugiura,S. Oda,T. Aoyagi, Y. Matsuo,R. Enomoto,T. Matsumoto,K. *et al.* 2007. Microfabricated Airflow Nozzle For Microencapsulation Of Living Cells Into 150 Micrometer Microcapsules. *Biomed Microdevices.* 9(1): 91-9.
[14] Chicheportiche D. and Reach G.. 1988. In Vitro Kinetics Of Insulin Release By Microencapsulated Rat Islets: Effect Of The Size Of The Microcapsules. *Diabetologia.* 31(1): 54-7.
[15] chrezenmeir,J. Gero,S L. Laue, C. Kirchgessner,J. Muller, A. A. Huls, *et al.* 1992. The Role Of Oxygen Supply In Islet Transplantation. *Transplant Proc.* 24(6): 2925-9.
[16] Sugiura,S. Oda,T. Izumida,Y. Aoyagi Y., Satake,M. Ochiai,A. *et al.* 2005. Size Control Of Calcium Alginate Beads Containing Living Cells Using Micro-Nozzle Array. *Biomaterials.* 26(16): 3327-31.

[17] Haeberle,S. Naegele,L. Burger,R. von Stetten,F. Zengerle, R. and Ducree J.. 2008. Alginate Bead Fabrication And Encapsulation Of Living Cells Under Centrifugally Induced Artificial Gravity Conditions. *J Microencapsul.* 25(4): 267-74