APPLICATION OF MICROFLUIDIC SPOTTING-BASED GENERATOR AND PLURONIC® F127 SOLUTION TO GENERATE COLLAGEN MICROBEADS MICROENCAPSULATION OF CELLS

S.B. Huang, H.C. Lee, S. W. Tsai and M.H. Wu*
Graduate Institute of Biochemical and Biomedical Engineering, Chang Gung University, Taoyuan, TAIWAN

S.B. Huang and H.C. Lee contributed equally to this work.

ABSTRACT

The cell microencapsulation has been used in a wide variety of biological or medical researches. There are many types of natural or synthetic polymers that have been used to encapsulate living cells. Among them, collagen is a frequently used hydrogel for the microencapsulation of cells mainly because of its excellent biocompatibility and biodegradability properties. Compared with the conventional cell microencapsulation methods, the utilization of microfluidic-based methodology can realize microbead generation in a more uniform and continuous manner. However, most of the proposed devices are not only technically demanding but might also be harmful to the encapsulated cells. These can hamper the subsequent application of cell-encapsulated microbeads. To tackle these issues, this study reports a spotting-based microfluidic device for continuous generation of cell-encapsulated collagen microbeads. The working principle is mainly based on the use of a pneumatically driven micro-vibrator to continuously generate tiny cells/collagen microdroplets in a thin oil layer. The temporarily formed collagen microdroplets soon sink into the sterile, and biocompatible Pluronic® F127 suspension (25 °C, 8% (v/v)) that stably maintains the shape of the collagen microdroplets during thermal gelation process. In this study, the size and uniformity of the collagen microbeads were regulated by the input flow rate of collagen suspension and the pulsing frequency of the micro-vibrator. Within the experimental conditions studied, the proposed method was able to generate collagen microbeads ranging from 73.9-353.4 μm in diameter with high uniformity (Coefficient of variation (CV): 6.8% -7.4%). Results also exhibited that the encapsulated 3T3 cells kept at a high cell viability of 96 ± 2%, suggesting that the proposed cell microencapsulation process is cell friendly. As a whole, the proposed device has opened up a route to generate collagen microbeads or microencapsulation of cells in a simple, continuous, controllable, uniform, and cell-friendly manner with less contamination.

KEYWORDS

Collagen, Microbeads, Microencapsulation, Microfluidic technology

INTRODUCTION

Cell microencapsulation is the technique of enclosing biological cells within matrix to form a microbead. Cell microencapsulation is widely used in various therapeutic applications like cell therapy or tissue repair. There are many types of natural or synthetic polymers that have been used to encapsulate living cells. Among them, collagen is a frequently used hydrogel for the microencapsulation of cells mainly because it is the major component of native extracellular matrix (ECM), and due to its excellent biocompatibility and negligible immunogenicity [1]. Reports in literature have demonstrated the use of collagen or its derivatives materials to encapsulate various cell types for different biomedical applications [2-3]. Compared with the conventional cell microencapsulation methods, the utilization of microfluidic-based methodology can achieve microbead generation in a more uniform and continuous manner. More recently, several microfluidic devices based on various working principles were actively proposed to perform microbead generation, or microencapsulation of cells [4]. However, these methods could be harmful to the encapsulated cells, or could lead to contamination due to the use of chemicals in the process. These can hamper the use of the cell-encapsulated microbeads in therapeutic applications. To generate cell-encapsulated microbeads in a simpler, continuous, controllable, cell-friendly manner with less contamination, this study reports a spotting-based microfluidic device for continuous generation of cell-encapsulated collagen microbeads. In this study, the working principle is mainly based on the use of a
pneumatically driven micro-vibrator, borrowing from our previous experience [5]. By regulating the collagen suspension loading rate, and the pulsatile frequency of micro-vibrator, the cell-encapsulated collagen microbead can be generated in a tunable manner. In this study, the device was designed and fabricated. Its performances on generating collagen microbeads were evaluated in terms of microbead size, uniformity, and microstructure of collagen microbead. In addition, the use of proposed method to perform microencapsulation of cells (3T3 cells) was demonstrated. Within the experimental conditions studied, the proposed method was able to generate collagen microbeads ranging from 73.9 to 353.4 µm in diameter with high uniformity (Coefficient of variation (CV): 6.8-7.4%). Moreover, the microstructure of collagen microbeads generated by the proposed method was compatible with the properties found in the collagen produced by the conventional methods. Furthermore, the encapsulated 3T3 cells kept at a high cell viability of 96±2%, suggesting that the proposed cell microencapsulation process is cell friendly.

MATERIALS AND METHODS

The photograph of the spotting-based microfluidic chip is shown in Fig. 1. It comprises an inlet for 3T3 cells /collagen suspension loading using a syringe pump, microchannel for the suspension flow, pneumatically-driven vibrator, and a needle. The assembly of the chip is illustrated in Fig. 2(a). Briefly, it consists of three microfabricated PDMS (Layer A, B, and C) layers, and a 30G needle connected to the hollow cylinder of pneumatic vibrator. The three PDMS layers were first permanently bonded with the aid of plasma oxidation treatment. This was followed by assembling with the needle to form a laminate structure with its cross-sectional view shown in Fig. 2(b). In this study, the working principle (Fig. 3) of continuous collagen microbead generation is mainly based on the use of a pneumatically driven micro-vibrator, borrowing from our previous experience [5]. Briefly, a compressed air was applied in the pneumatic chamber that leads to the deformation of the PDMS membrane (also refer to Fig. 2(b)). Such a deformation forces the hollow cylinder and also the connected needle to move downwards (Fig. 3(b)). The release of the pressure causes the translocated needle to restore to its original location (Fig. 3(c)). By the above repeated spotting movements, the proposed chip was able to continuously generate tiny 3T3 cells/collagen microdroplets into the thin oil layer (Fig. 3(c)). Due to their higher volumetric density than the corn oil used in this study, the temporarily formed collagen microdroplets soon sink into the sterile, and biocompatible Pluronic® F127 suspension (25 °C, 8% (v/v)) (Fig. 3(d)) that stably maintains the shape of the collagen microdroplets due to its surfactant property. The Pluronic® F127 suspension with the generated 3T3 cells/collagen microdroplets was then kept at a 37 °C condition for 20 min. Under the thermal condition, the collagen gelation occurs and thus becomes solid microbeads (Fig. 3(e)). After that, the generated 3T3 cells/collagen microbeads were placed in a fresh culture medium for cell culture (Fig. 3(f)). In this study, the size and uniformity of the collagen microbeads are regulated by the input flow rate of the collagen suspension and the pulsing frequency of the micro-vibrator. In order to investigate the quantitative relationship between them, the following experiments were performed. Briefly, the pneumatic pressure and pulsation frequency of the micro-vibrators were set at 10 psi and in the range from 5-15 Hz, respectively. The collagen suspension loading rate was set in the range of 0.2-8.5 µl min⁻¹. The determination of these conditions is based on our preliminary study to ensure the optimal operation stability. The collagen microbeads generated at these investigated conditions are collected and the images of the beads are then captured using a digital camera-coupled microscope. The sizes of the microbeads are then measured using an image analysis software (SimplePCI version 5.2.1, Compix Inc.) to determine the diameter of the microbeads. For the evaluation of microbead uniformity, the images (about 100 microbeads/image) are then analyzed to evaluate the size (in diameter) distribution of the collagen microbeads. The coefficients of variation (CV) (the ratio of the standard deviation to the mean value of the diameter) are then obtained from this data. To ensure the cell microencapsulation process is cell-friendly, the cell viability of the encapsulated 3T3 cells was evaluated using a Live/Dead fluorescent dye kit, and a fluorescent microscopic observation.

RESULTS AND DISCUSSION

In the proposed device, the size of resultant collagen microbeads is mainly determined by the loading rate of collagen suspension and the frequency of the spotting movements. To find out the operation conditions, by which the generated collagen microbeads are relatively uniform (e.g. the
CV value <10%), experiments were carried out. Fig. 4 revealed the quantitative relationship between them. It is not out of our expectation that the conditions of lower suspension flow rate and higher frequency vibration can contribute to the microbeads with smaller size. As a whole, the collagen microbeads with the size ranging from 73.9 to 353.4 µm in diameter can be generated at the suspension flow rate and vibration frequency ranges of 0.2–8.5 µl/min and 5–15 Hz, respectively. Besides, the uniformity of the collagen microbeads is also an important technical issue. To exactly examine this, the uniformity of collagen microbeads formed under above conditions was evaluated and was expressed as CV values. Results (Fig. 4) showed the CV values of the collagen microbeads generated under the experimental conditions explored was in the range of 6.8-7.4%, indicating relatively uniform microbeads can be produced by the proposed method. Fig. 5 (a) and (b) exhibited the microscopic images, and the size distributions of collagen microbeads generated under the suspension flow rate of 0.2 and 6.0 µl min⁻¹ and the pulsation frequency of vibrator of 15 Hz and 10 Hz, respectively. The resultant sizes of microbeads were 73.9 µm and 253.6 µm in diameter, respectively. Moreover, the calculated CV range is lower than 8%.

In this study, the feasibility of using the proposed method for encapsulating living 3T3 cells in collagen microbeads was successfully demonstrated as shown in Fig. 6. Figure 6(b) showed the fluorescent microscopic observation of the cells encapsulated in the microbeads, in which the green and red dots represent the live and dead cells, respectively. The cell viability estimate by counting the live (green) and dead (red) cells. Within the experimental conditions investigated, the calculated results showed that the cell viability for the 3T3 cells encapsulated in collagen microbeads (Fig. 6(b)) was as high as 96±2%. As a whole, it demonstrated that the proposed method is not only able to achieve the collagen microbead generation in a simple, controllable and uniform manner, but it also offers a cell-friendly way to microencapsulate living cells.

CONCLUSIONS

This study reports a new microfluidic device with a pneumatically-driven spotting-based generator for continuous generation of cell-containing collagen microbeads. Overall, the proposed method was proved to be able to generate the collagen microbeads with sizes ranging from 73.9 to 353.4 µm in diameter with the CV from 6.8% to 7.4%. Moreover, its application for the microencapsulation of 3T3 cells in collagen microbeads has also been demonstrated with high cell viability (96 ± 2%).

REFERENCES


CONTACT

*M.H. Wu, tel: +886-32118800#3213; mail: mhwu@mail.cgu.edu.tw

![Figure 1: A photograph of the microfluidic chip device consisting of pneumatically driven vibrator.](image-url)
Figure 2: (a) Assembly of the microfluidic chip for collagen microbead generation, and (b) a cross-sectional view of the structure.

Figure 3: The collagen microbeads generation process by using the microfluidic device.

Figure 4: The size evaluation of collagen microbeads generated at varied collagen suspension flow rates and pulsation frequencies of the pneumatic vibrator.

Figure 5: Images of collagen microbeads with an average diameter of (a) 73.9, and (b) 253.6 µm, and their size distributions.

Figure 6: (a) The CCD image of the 3T3 cells within 150 µm collagen microbeads. (b) Fluorescent images of 3T3 cells encapsulated in collagen microbeads. Green and red dots represent live and dead cells, respectively.