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Development of Porous PCL Based Microcarrier

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***Abstract*— Polycaprolactone (PCL) has been used for cell cultivation due to its biocompatibility. Emulsion solvent evaporation method is widely used in fabrication of sphere base microcarrier. The fabricated microcarrier can be further modified base on the preferences toward the ideal micocarrier. The significant characteristics of ideal microcarrier are the uniform size of microcarrier, density and surface properties of the microcarrier. The uniform size of microcarrier is important to determine the average viable cell in culture. The ideal density of microcarrier should be slightly higher than the density of water. This is to ensure the fabricated microcarrier is in suspension in culture medium with minimal agitation exert. The surface of microcarrier should contain a functional properties either the surface charge or the microbiological component that may attaract cell to adhere and proliferate on the microcarrier. In this study, microcarrier were fabricated with micropores structure, this is to provide larger surface area per volume with intention to increase number of cell proliferation on the microcarrier. From the result, size distribution of the fabricated microcarrier and the size of pore generated on microcarrier were affected by most of the parameters tested such as sterring speed, PVA concentration, camphene concentration, temperature and ratio between PCL and solvent. Optimum pore size was generated at 20% camphene concentration with the average size of 11.74 µm which is conducive for cells to attached and populated well within the pores. The surface properties of developed porous can be improved for potential application in cell culture research and development, as well as in tissue engineering.**

***Keywords*— halal; microcarrier; cell culture; porous; polycaprolactone.**

I. INTRODUCTION

In recent years, microcarrier system has been established as a cell carrier in mammalian cell culture and tissue engineering. Various types of microcarrier available in the market with each of them carry different properties in order to cater the variation in cell behavior and traits. Study reported that microcarrier with porous structure attained more efficient cell proliferation as compared to non-porous microcarrier [1]. Polycaprolactone (PCL) is a biodegradable, biocompatible and semi-crystalline polymer that has melting temperature at 60 ^{o}C [2]. It has been widely used in medical and tissue engineering as a biomaterials due to its good properties. PCL's approval from the US Food and Drug Administration (FDA) promise a vast advantage of this material in tissue engineering and medical uses [3]. In a previous study a smooth surface PCL microcarrier with surface modification has been developed and tested with few cell line and the finding shows PCL microcarrier is compatible as a cell carrier [4]. From the microscopic point of view, microcarrier could performed better as a cell carrier if it is designed to be porous.

There are several methods for the fabrication of porous microcarrier including oil/water emulsion solvent evaporation, spray drying technique and solution-enhanced dispersion method [2]. In this current study, emulsion solvent evaporation method in the presence of camphene was used to prepare the porous microcarrier. Camphene is a porogen that is widely used as a source of fragrance in the market and camphene has been used in ceramic scaffold as a processing tools to generate pores[5].

The aim of this study is to screen parameters that affect the fabrication of PCL-based porous microcarrier. One-factor-at-a-time (OFAT) method was implemented and the parameters selected were camphene concentration, PCL to dichloromethane ratio, polyvinylalcohol (PVA) concentration, stirring speed, stirring time and stirring temperature.

II. Materials and Methods

A. Chemicals and reagents

Polycaprolactone (PCL) (Mn = 45,000) supplied by Sigma-Aldrich (St. Louis, MO, USA) was used to fabricate the porous microcarrier. Camphene, dichloromethane (DCM) and polyvinyl alcohol (PVA) were purchased from Merck Milipore (Darmstadt, Germany), phosphate buffer saline (PBS) solution were purchased from Sigma-Aldrich (St. Louis, MO, USA).

B. Preparation of porous PCL microcarrier

Microcarriers were prepared by dissolving polymer matric (PCL) in the organic solvent (dichloromethane). Porogen (camphene) that assist in the formation of pore in the microcarrier were also dissolved in dichloromethane. The two dissolved solution (PCL and camphene) were mixed together and was stirred for another 3 hours. Surfactant (polyvinyl alcohol; PVA) was dissolved in 90 ml deionized water and stirred on hot magnetic stirrer at 60 ℃. Prior to commenced of the solvent evaporation process, PVA solution was placed in the fume hood and stirred at 250 rpm using overhed stirrer. The mixture of PCL/camphene was then added to PVA solution dropwise. The resulting emulsion was continue to for 6 hour at certain temperature. The prepared microcarriers were recover by using a sieve tray and washed with deionized water several time. The microcarriers were dried in a 40 ℃ oven overnight. The microcarriers were prepared by varying six parameters as listed in Table I following the one-factor-at-a-time (OFAT) method.

TABLE I
Parameters Used in OFAT Experiments

Parameter	**Range**	**Responses of the study**
Stirring speed, rpm	150 - 350	Pore size and particle size
PVA concentration, % w/v	0.5 - 2.5	
Camphene concentration, % w/v	0 - 60	
Temperature, ℃	25 – 45	
Stirring time, hour	4 – 8	
PCL : DCM , % (w/v)	5 - 25	

C. Charaterization of porous microcarrier

The size distribution of the microcarriers was determined by a BT-9300H Particle Size Analyzer (Bettersize Instruments, China). Prior to pore characterization, the microcarriers were coated with carbon on the metal slab by using a carbon coater (Polaron CC7650). Optical microscopy images of the microcarriers were taken by selecting 9 arbitrarily selected areas on the microcarrier, followed by image analysis using Image J® software [1]. Electron microscopy images were viewd and captured with a JEOL JSM 5600 scanning electron microscope.

III. Results and Discussion

A. Effect of PVA concentration

Fig. 1 ilustrate the outcome from the variation of the PVA concentration on the size distribution of the microcarrier particles. At PVA concentration of 0.5% to 1.0%, slight decrese in the size of particles were observed. Increased in PVA concentration may reduced the interfacial tension of the solution thus lead decreased in the size of droplets [5]. At this condition the emulsion was stabilized by the surfactant through imparting thin layer around the droplet, therefore avoiding coalescence and producing smaller particles [9]. Further increase in PVA concentration from 1.0 % to 1.5% causes the microcarrier particle size to change from ~112 µm to ~138 µm. This is in agreement with condition that report by Rafati et al. [6] and Kemala et al. [7]. Two opposing effects may occur at this point where the interfacial stabilization was enhanced by surfactant thus reduced the microcarrier size. At the same time, increased in the PVA concentration, may lead to increase of the viscosity of the surfactant, therefore lead to increased in microsphere size [8]. Hence, increase in particle size at 1.5% PVA can be concluded that the effect of surfactant viscosity might overcome the stabilizer effect of the surfactant. Futhermore, by increased the PVA concentration up to 2.5%, the particle size was reduced due to the stabilization of interfacial force thus reducing the size of the particles.

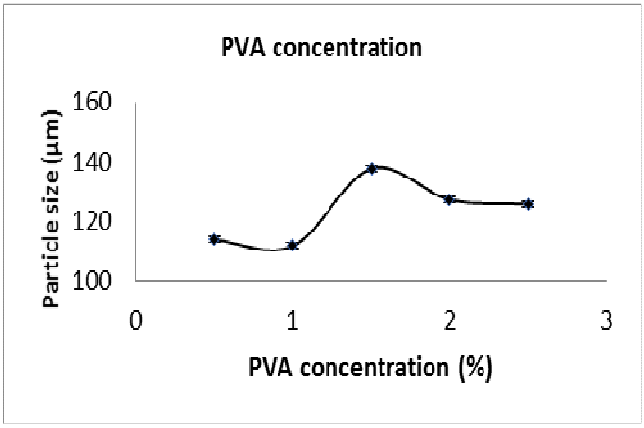


Fig. 1 Effect of PVA concentration on the particle size

Fig. 2 showed the effect of PVA concentration on the size of pores. Varying the PVA concentration from 0.5% to 1.5%, it was observed that the size of pores were increased. High PVA concentration result in smaller particle size but apparently increase the size of pores [9]. High PVA concentrations, the diffusion of the dichloromethane become harder thus prolong the coarsening time of microcarrier beads and lead to larger pore size.

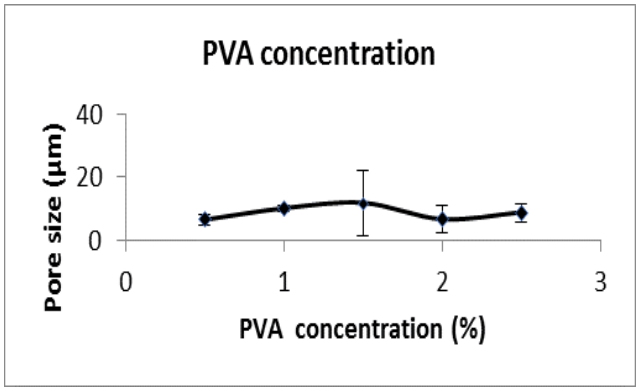


Fig. 2 Effect of PVA concentration on the pore size

B. Effect of camphene concentration

Additional of small molecule which is camphene into the raction mixture, posed a vital roles in generating pore channels network within the PCL microcarrier. The interesting properties of camphene that help in generating the pores are the solidification temperature of camphene at around 40 ℃ and excessive vapor pressure that makes it easier to sublime under ambient temperature. In the process of solidification where solvent evaporated, camphene was separated (sublime) from PCL thus form the interconnected pores within the microcarrier. The result shows that as camphene concentration increases, the size of pore on the PCL microsphere also increases (Fig. 3). Further increase in the porogen concentration, the solubility of polymers decreases therefore increased the phase separation process. Larger size of pore were formed due to the separation of the phase area take longer time to grow thus, coalesce with each other [10].

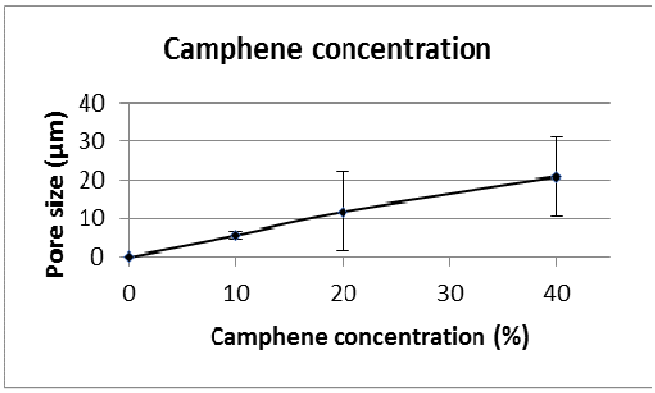
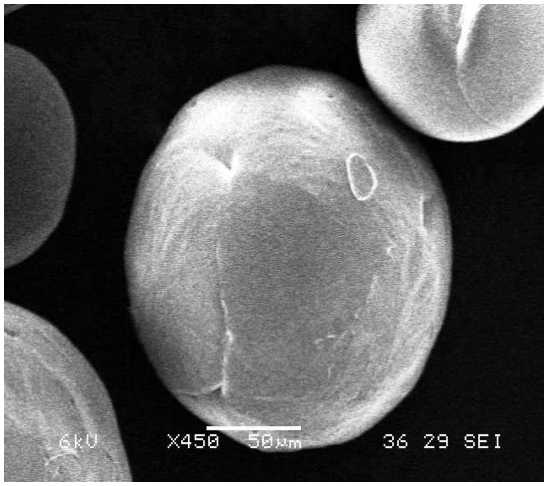
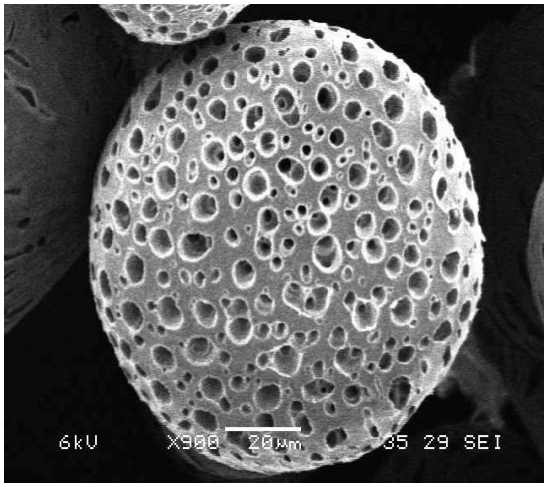


Fig. 3 Effect of camphene percentage on pore size

Fig. 4 shows the morphology of the microcarrier with different camphene concentrations. At the highest concentration studied (60% w/v camphene concentration), the structure of the microcarrier is not stable and collapsed due to the very big pores. The optimum condition for pore size formation was found at 20% w/v camphene concentration with even distribution of pores on the surface and the microcarriers have larger pore size which could enable cells to penetrate the pores and populate inner surface of the microcarrier.



(a)



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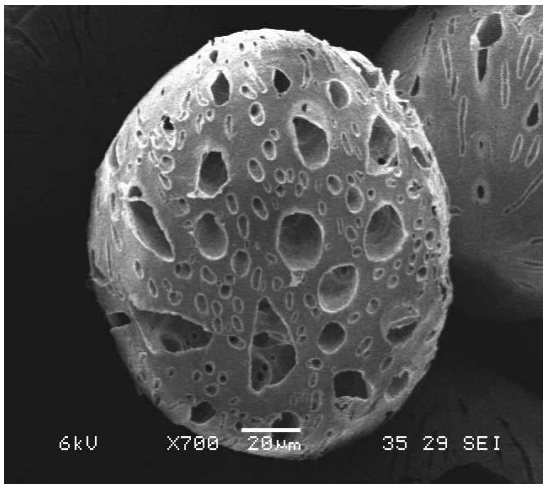
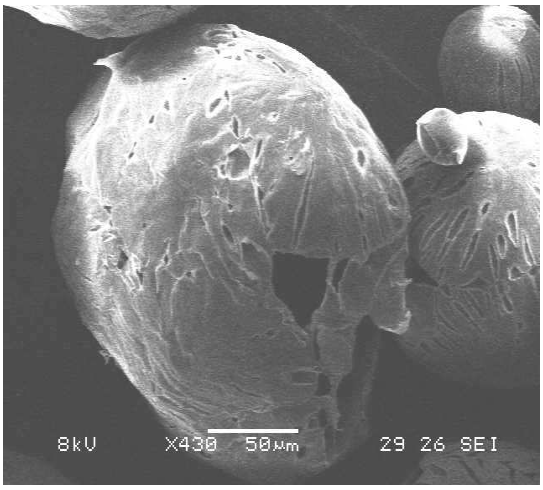
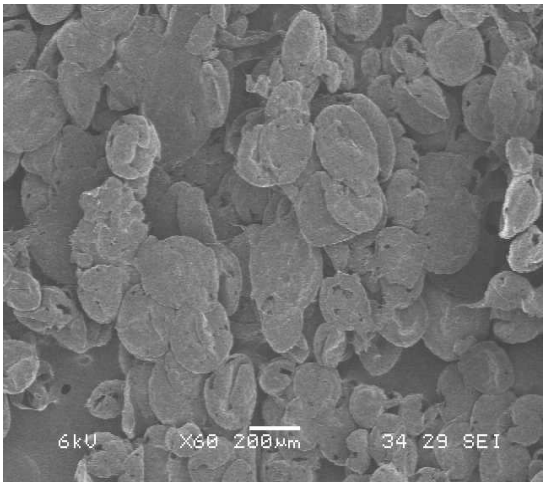


(c)



(d)



(e)

Fig. 4 SEM morphology of the PCL micro-carriers. The concentration of camphene was varied: Camphene ratio (a) 0%, (b) 10%, (c) 20%, (d) 40% and (e) 60%.

C. Effect of PCL:camphene ratio

Result in Fig. 5 shows that the particle size is increased as the PCL to dichloromethane (DCM) ratio increased. Heiskanen et al. [11] address that the size of microcarrier increased as the concentration of polymer solution increase. The viscosity of the polymer solution was expected to contribute to this relationship. Therefore, with the same mixing intensity, larger droplets were produced as PCL concentration increases [12].

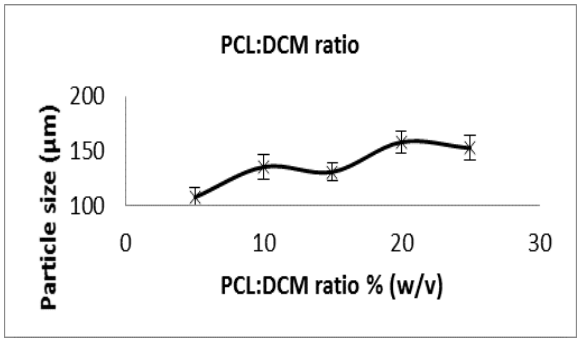


Fig. 5 Effect of PCL to DCM ratio on micro-carrier particle size

D. Effect of stirring speed

The stirring speed is expected to have larger influenced towards the microcarrier particle size . The increase in agitation from 200 rpm to 350 rpm causes the particle size to decrease from an average size of ~ 149 µm to ~ 96 µm. However, at a low agitation of 150 rpm large droplets were formed and did not solidify. This is due to the reduced in diffusion ability of the solvent that caused by the low intense of agitation force to disperse the droplet thus, lead to the non-solidify droplet. As reported Heiskanen et al. [13], as the agitation speed were decreased from 450 to 500 rpm, the microcarriers size were also decreased. Higher stirring speed provides high shear forces and reduced the coalescence in the mixture led to decrease of the microcarrier size.

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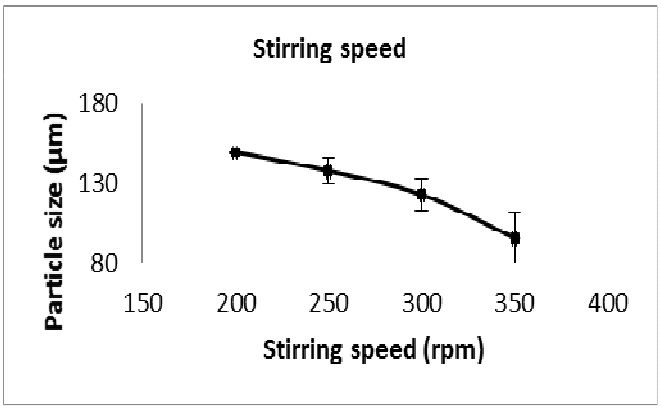

Fig. 6 Effect of stirring speed on microcarrier particle size

E. Effect of Stirring temperature

Fig. 7 and Fig. 8 show the effect of fabrication temperature on microcarrier particle size and pore size, respectively. As the temperature increased, the particle size and the pore size decreased. As stated by Yang et al., the fabrication temperature affect the morphology of biodegradable polymeric microcarrier fabricated by double-emulsion method for temperature between 4 ℃ and 29 ℃ [11]. Vicousity of the emulsion is lower at higher temperature, therefore, it is to break up the emulsion into smaller droplets.

Zhang et al. [1] reported that as they increase the temperature from 40 ℃ to 60 ℃, the pore size of microspheres decreased. The polymer chain became more flexible as the temperature was increase towards the melting poin of PCL. As a result, the mixture solution viscosity became low and led to the agglomeration.

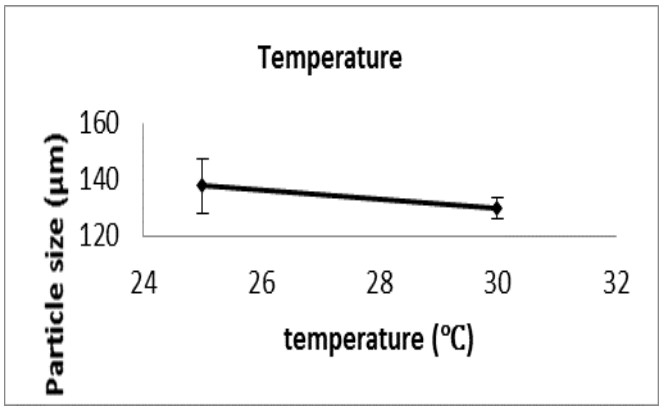

Fig. 7 Effect of temperature on microcarrier particle size

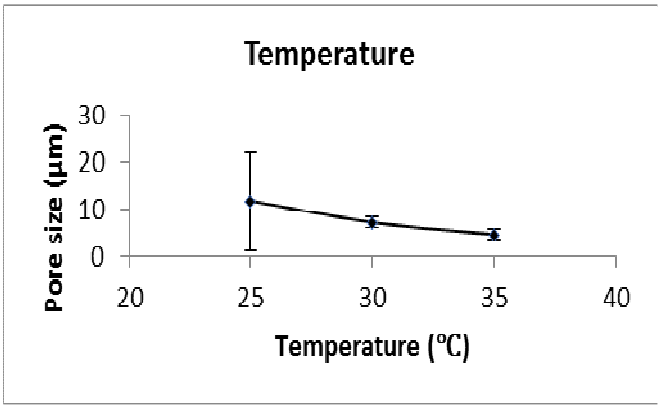

Fig. 8 Effect of temperature on microcarrier pore size

F. Effect of Stirring time

Another parameter that not only affect the particle size but also the pore size is the stirring time. Fig. 9 and Fig.10 showed the effect of agitation time on particle size and pore size, respectively. Both microcarrier size and pore size showed decreasing trend as the stirring time increased; the stirring time increased from 6 hours to 8 hours, the particle size and pore size decreased (Fig. 9 and Fig. 10, respectively). However, solidification of the microcarrier did not occur when stirring time was less than 6 hours. At 6 hours agitation time, the recorded particle size and pore size were ~138 µm and ~12 µm respectively. It is also found that the pore size also decreases as the stirring time increases.

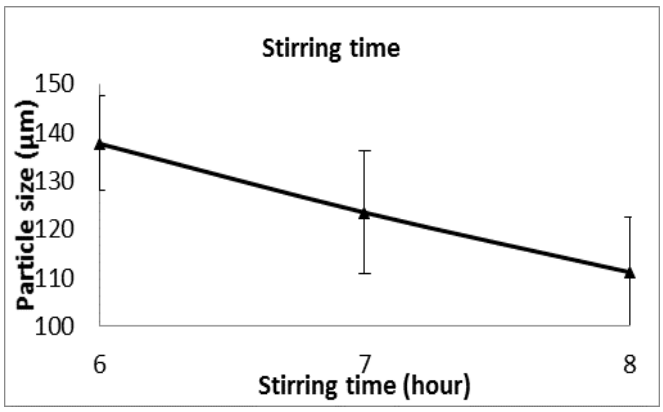

Fig. 9 Effect of stirring time on micro-carrier particle size

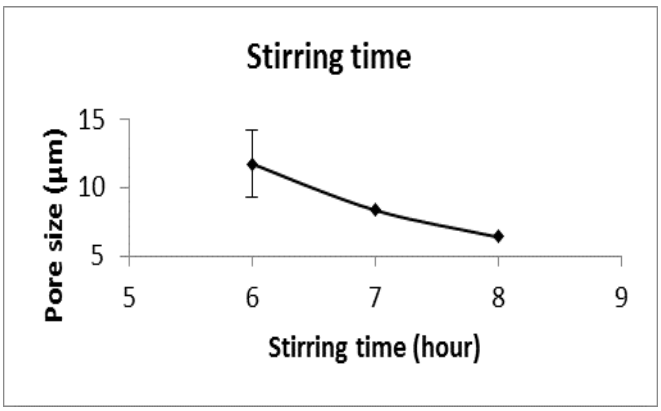

Fig. 10 Effect of stirring time on micro-carrier pore size

IV. Conclusions

Porous PCL has been successfully generated in the presence of camphene using the solvent evaporation method. Parameters such as stirring speed, PVA concentration, camphene concentration, stirring temperature and stirring time which have effect on the particle size and pore size were identified. Camphene concentration has vital effect on the pore size of the microcarrier. Camphene at the concentration of 20% (w/v) resulted in average pore size of 11.74 µm which allow cells to migrate into the pore channel and proliferate in interior space of microcarrier.

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