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Effect of powder particle size on solvent free membrane for 3D hybrid scaffold structure

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Abstract: Solvent free membrane fabrication is a powder based method that can generate porous structure without used of organic solvent that can harmful to cell both *in vitro* and *in vivo*. In this paper, the effect of powder size on membrane morphology and surface wettability were investigated. Two different Poly (ε-caprolactone), (PCL) particle sizes of powder which are 100 µm and 500 µm were used. Powder was spread on top of water and by heating with laboratory oven in was melted down. After 10-15 minutes, it was fully melted and membrane can be obtained after cooled down. The powder was really affect morphology of membrane both macroscopic level and microscopic level. For macroscopic level, 500 µm membrane for different surface texture between water contact side and air contact side which air contact side gave smoother surface. On the other hand, for microscopic level, 100 µm showed more porous structure. Lastly, by further investigate the surface properties using contact angle, it confirmed that there is no significant between two different sides of 100 µm membrane but for 500 µm membrane it showed different manner because the contact angle results that was around 10 degree different between two sides.

Introduction

An integration between additive manufacturing (AM) techniques and biomaterials has been used to serve tissue engineering applications because it shows ability to construct and organize complex structures (Melchels et. al (2012)). For solid scaffold based tissue engineering, there is many Additive manufacturing approaches to make scaffold using biodegradable polymer which are selective laser sintering, (SLS) (Liao et. al (2011)), fused deposition modeling, (FDM) (Zein et. al (2002)) and stereolithography, (SLA) (Santos et. al (2013)). Biodegradable polymers such as polycaprolactone, (PCL) , Poly(L-lactic acid), (PLLA) (Tan et. al (2005)) and poly(ethylene glycol), (PEG) (Melchels et. al (2012)) are common materials that have been used with AM techniques. Nowadays, the new trend of solid scaffold free cell base has been introduced to AM techniques recently. The breakthrough technology which is called "bioprinting" has been promise to product novel soft scaffold structure (Mironov (2006)). The bioprinting used hydrogel and cell which combine into the mixture to print so it will be the gel like scaffold instead of hard scaffold as using conventional AM methods. Hydrogels can provide appropriate environments for cell to growth. In bioprinting, hydrogels need to have specification of properties which are have no immunity effect, able to promote suitable surrounding for cellular activity, and suppose to promote

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migration and perforation of cell (Murphy et. al (2013)). However, hydrogels contain more than 90% of water so it still limited to support 3D structure due to lack in mechanical strength (Melchels et. al (2012), Murphy et. al (2013)). Thus, the hybrid structure between solid scaffold and soft gel scaffold can be one of possible solutions for this problem because this structure can combine suitable environment for cell and good mechanical properties (Melchels et. al (2012)). The hard structure for this hybrid scaffold can be fabricate using AM technique or can be in the form of thin film like membrane which can benefit the properties control in each single layer. In order to make membrane, there are many methods to fabricate it included solution casting (Tiaw et. al (2007), Yang et. al (2002)), freeze extraction (Gaona et. al (2012)) and elctrospinning (Ding et. al (2004), Mitchell et. al (2011)). All of these methods cannot promise to create porous structure without involve with organic solvent which can be harmful to cell and tissue. Thus, solvent free method was discovered in order to avoid the used of toxic chemicals. Advantages of this method are not only organic solution free but also fast and simple. By deposit powder on top of water surface and insert the heat into system, membrane can be easily formed. In this paper, we further studied this powder based method by comparing between two difference powder particle sizes. Membrane morphology and surface wettability was investigated in order to find proper structure and condition to serve as solid based scaffold for 3D hybrid structure.

Materials and methods

Poly(ε-caprolactone) membrane fabrication

Poly(ε-caprolactone) powder (CAPA® 6501, Molecular weight: 50kDa) was purchased from Solvay Interox, UK. The material density of poly(ε-caprolactone) is 1.1 g/cm^3. The melting point is around 60 °C. The average particle size is about 100 μm. Another size of PCL powder is 500 μm PCL powder which was purchased from Perstorp. First, 30 ml de-ionized water was poured into a 10 cm diameter round glass dish at room temperature. Then, 0.1 gram of 100 μm PCL powder was dispensed gently onto the water surface. For 500 μm PCL powder, 0.7 gram was required to be sufficient to cover the whole water surface. Third, the glass dish was shaken slowly to confirm the equal spreading of powder on to the water surface. A rubber bulb was used to in order to get rid of excess powder. Next, the glass dish was put onto a laboratory heater (Thermolyne Cimarec® 1, Thermo scientific, USA) and the level of heater was set at level 2 through all the experiment. After 10 minutes for 100 μm powder and 15 minutes for 500 μm powder, the heater was turn off and the glass dish was put down from the heater. Finally, the glass dish was cooled down at room temperature. After about 5 minutes, a solid white membrane appeared on top of water surface.

Membrane Characterization

Membrane morphology

Membranes were cut into 10x10 mm rectangular shape then light microscopy (Olympus, CKX41) was used to examine the morphology of microstructure of for both membranes. The magnification was fixed at 4 times.

Surface wettability

Water contact angle for both membranes were analyzed by using Theta Optical Tensiometers (Helsinki, Finland). Membranes in the size of 10x10 mm were placed on glass slide. Distilled water was used as wetting medium which each drop contain a volume of 5 μL. A single drop of water was directly contact to the surface of membranes. The shape of a droplet was recorded by using digital camera which was controlled via Attension Theta software.

Result and Discussion

First of all, by using different size of powder, the fabrication time was also different. For the same size of petri dish, more amount of bigger powder need to cover all the surface area. Therefore, for bigger powder, more energy requires to melt it down. From this fabrication method, membrane showed two side of surface differentiation which are water contact side and air contact side. The comparison on macroscopic of both membranes was shown in figure 1. It demonstrated that the 100 µm membrane surface was smoother than the 500 µm membrane surface. Moreover, both sides of 100 µm membrane looked similar (figure 1a and 1b) in macroscopic view but for 500 µm membrane a difference between two sides of surface are very clear. As shown in figure 1c and 1d, the air contact side is smoother than the water contact size.

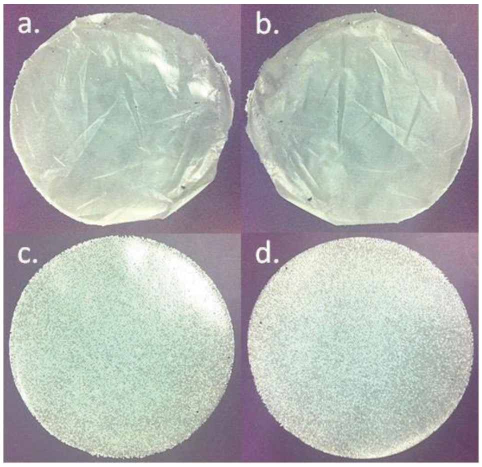

Figure 1. Macroscopic view of (a) 100 µm membrane air contact side (b) 100 µm membrane water contact side (c) 500 µm membrane air contact side (d) 500 µm membrane water contact side

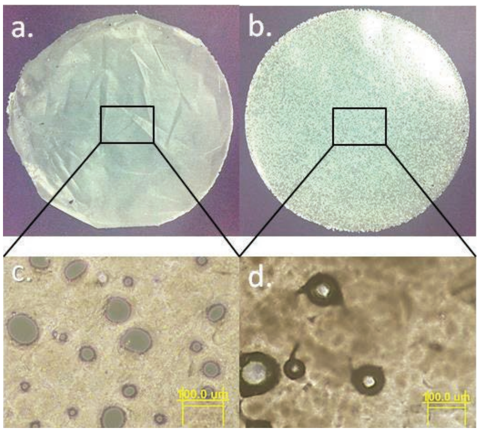

Figure 2. Macroscopic view of (a) 100 µm membrane (b) 500 µm membrane, Microscopic using light microscope magnification at x4 (c) 100 µm membrane (d) 500 µm membrane

In figure 2, it showed the microscopic view of both membranes. From figure 2c and 2d, they showed that in the same area, more pore distribution on 100 μm membrane compared to 500 μm -- membrane. This also resulted from the different in the average particle size of powder. Using bigger powder seems to obtain less number of pores and showed less distribution.
Another significant observation is that from water contact angle testing result in table 1, for two sides (air contact side and water contact side) of 100 μm membrane static contact angle results were similar, however, for 500 μm membrane results did not express in the same manner. From table 1, the results showed that the air contact side was more hydrophobic compared to water contact side. There is a report stated that surface properties such as roughness and hydrophobicity effect to cell behavior on the surface (Yang et. al (2013)). Therefore, the morphology of surface and hydrophilicity are important properties for cell adhesion should be further investigated.

Table 1. Static contact angle results of membranes

	Contact angle	
	Water contact side	Air contact side
100 μm membrane	71.60 ± 1^o	72.60 ± 1^o
500 μm membrane	72.50 ± 2^o	81.58 ± 3^o

Conclusion
In this paper, the effect of powder particle size to membrane fabrication and structure was presented. Different size of powder requires different amount and also different in time to fabricate the membrane. Moreover, size of powder also affected surface morphology of membrane and surface hydrophobicity. As shown from results, bigger particle sizes leaded to different in surface morphology between two sides of surface which are water contact side and air contact side compared to smaller size. Not only morphology but also hydrophilicity of surface was affected by the size of powder. The results showed that air contact side was more hydrophobic and also smoother which can be clearly seen in 500 μm membrane. These membranes made by using polycaprolactone which is biocompatible and biodegradable so it is useful for tissue engineering and biomedical applications. Thus, the next step is to investigate cell behavior on the surface and the construction of hybrid system between membrane and hydrogel from bioprinter in order to achieve 3D organized structure for tissue engineering hybrid scaffold.

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