NANOFIBROUS SURFACE PATTERNING USING NANO-MESHED MICROCAPSULES INDUCED BY PHASE SEPERATION ASSISTED ELECTROSPRAY

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ABSTRACT

This is the first report on a nanofibrous surface patterning process using nano-meshed polymer microcapsules for biological applications. The nano-meshed microcapsules were formed using a method named phase separation assisted electrospray. Features of the nano-meshed microcapsules were found to be tunable by adjusting process conditions. Contrary to non-woven mats formed of continuous nanofibers via conventional electrospinning, nano-meshed microcapsule reported herein enabled a single step nanofibrous surface patterning by electrostatic focusing following the microcapsule formation. The biodegradable polylactic acid made microcapsules were patterned onto a glass substrate with up to 500μm resolution and human hepatocyte cells were cultured on the patterned areas to confirm biocompatibility of the microcapsule.

INTRODUCTION

Currently nanofibrous surface modification has been attracting a lot of attention, especially in biological and medical applications. Nanofibrous surfaces composed of biocompatible tiny polymer fibers ~φ100nm can effectively interact with cell surfaces and promote cell proliferation [1]. Among several methods to fabricate nanofibrous surfaces, electrospinning has been widely explored because of its simplicity and versatility [2]. In electrospinning, a polymer solution is ejected from a charged nozzle toward a target electrode substrate of opposite polarity (Fig.1). After being ejected from the nozzle tip the polymer solution is elongated and simultaneously splits into many thinner fibers due to external electrostatic attractive forces and internal electrostatic repulsion. Thus, a nanofiber mesh is obtained on the target electrode.

For tissue engineering applications, micro-patterned nanofibrous surfaces are highly required to guide cell proliferation in defined configuration. However, direct micro-patterning of nanofiber meshes formed by electrospinning is of great difficulty in principle because of the fibers' continuous form. Although there are some reports on patterning electrospun nanofibers on a substrate by patterning electrodes on the target substrate, the patterned and un-patterned areas are not fully separated due to the fibers' continuity.

In electrospinning, the viscosity of the polymer solution affects the morphology of the product created. By decreasing the viscosity of the polymer solution, nanofibers gradually thins until a point where the fiber starts to break up and particulate. At such sufficiently low viscosities, micro-particles below φ1μm are formed – a process called electrospraying differentiated from electrospinning. The morphological change of the electrospinning products depending mainly on the solution conditions has been thoroughly investigated to obtain uniform nanofibers [3]. Compared to the explosive increase in researches in recent years on electrospun nanofibers, a lot less attention has been paid to the microparticle formation process since mere microparticle can be produced using other methods.

One foggy morning in spring 2006, one of the authors M.F. found porous particulated structures in the products of electrospun nanofibers. Since then, we have extensively studied the formation process of the porous structures, and found the ambient humidity is the key factor among numerous parameters for the porous particle formation. By fine tuning the solution conditions and the ambient humidity, we can now control the morphology of the products – nanofiber, particle-nanofiber complex, normal particle and porous particle (Fig.2).

In this report, the formation conditions of the porous particle named "nano-meshed microcapsule", was investigated, and a novel direct surface patterning method of the nano-meshed microcapsule using electrostatic focusing was described. Finally, the biocompatibility of the patterned surface was examined by culturing human hepatocyte cells for future tissue engineering applications.

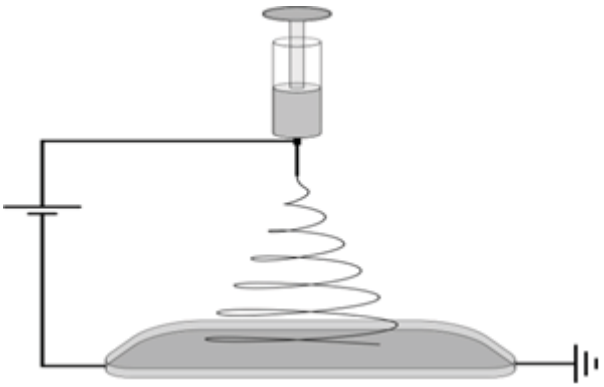

Figure 1: Schematic of the electrospinning process. A polymer solution is fed to a metal capillary by a syringe. A high voltage is applied to the capillary, forcing the polymer to eject towards the target electrode

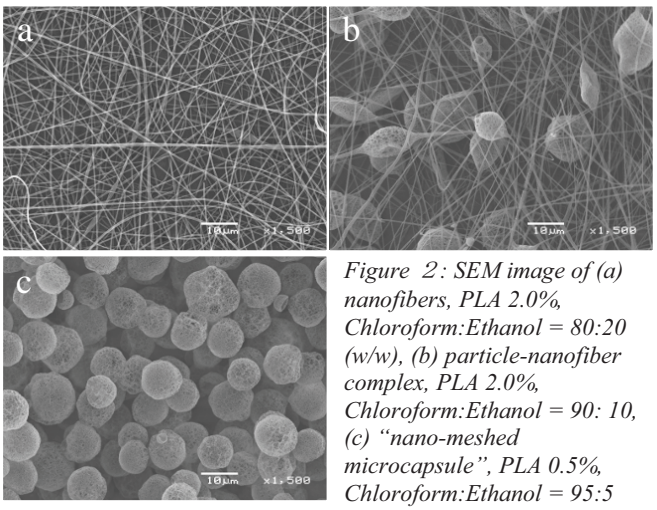

Figure 2: SEM image of (a) nanofibers, PLA 2.0%, Chloroform:Ethanol = 80:20 (w/w), (b) particle-nanofiber complex, PLA 2.0%, Chloroform:Ethanol = 90: 10, (c) "nano-meshed microcapsule", PLA 0.5%, Chloroform:Ethanol = 95:5

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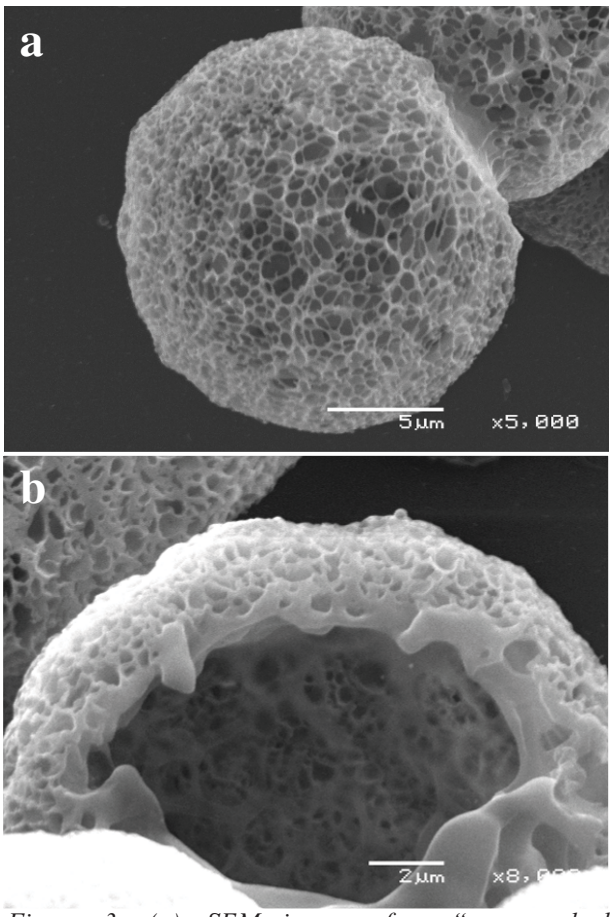

Figure 3: (a) SEM image of a "nano-meshed microcapsules" induced by phase separation assisted electrospray (b) Cross-section of a microcapsule cut with an excimer laser: The edge was slightly deformed due to heat generated by excimer laser ablation.

FORMATION OF THE NANO-MESHED MICROCAPSULE

In electrospinning or electrospraying, there are numerous parameters needed to be adjusted to control the final product. Among these parameters one of the most critical must be the composition of the polymer solution. Usually polymer solutions prepared for electrospinning or electrospraying are a mixture of polymer and solvents.

In this report, the polymer solution is constituted of 1) polylactic acid (PLA): a synthetic biodegradable polymer commonly used for biomedical applications, 2) chloroform: a volatile liquid used as a solvent, 3) ethanol: used as a secondary solvent to adjust the viscosity of the solution. By changing the ratio of the above three constituents, a wide range of morphologies can be created ranging from nanofibers to micro particles. It has been reported that the key factors of polymer solution that influence the formation of various morphologies are viscosity, elasticity, conductivity, and surface tension. When ratio of constituents is altered, all of the above properties of the solution are changed resulting in different morphologies for final products.

Nanofibers ϕ100~200nm was formed with polymer solution constituted with an excessive amount of ethanol (Fig.2a). When the ratio of ethanol was decreased, nanofibers started to contain irregular particles (Fig.2b).

Further decreasing of ethanol along with the decreasing of polylactic acid resulted in the disappearance of fibers and the formation of uniform sized microparticles. We found the surface of the microparticle became porous (Fig.2c), when the process was taken place in high ambient humidity. The porous microparticle, named "nano-meshed microcapsule", was found to have a hollow spherical shell composed of nanofibers ϕ100~200nm. The nanofibers formed penetrating microporous structure in the shell (Fig.3).

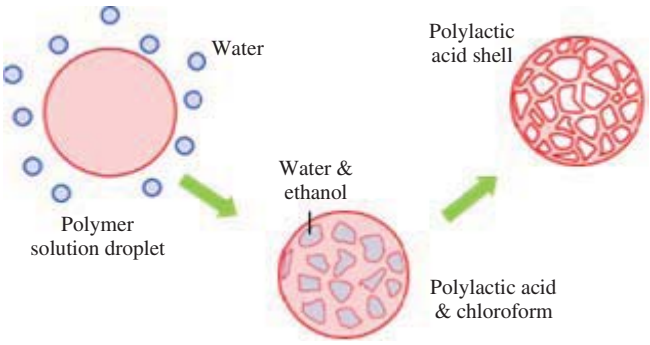

Figure 4: Schematic representation of the formation process of the nano-meshed microcapsule induced by phase separation assisted electrospray

The possible mechanism of the nano-meshed microcapsule formation is as follows (Fig.4): 1) the polymer solution jet electrosprayed from the nozzle breaks up into tiny droplets due to instability of the surface and electrostatic repulsion, 2) The droplet subjected to high ambient conditions, and water vapor condenses on the surface of the microparticle causing phase separation between the water and solvent phases, 3) the droplet of the polymer solution expands to a spherical shell due to electrostatic repulsion, 4) the water and solvent rapidly evaporates, leaving the polymer to remain to form a nano-meshed structure.

CONTROL OF THE DIMENSIONS OF THE NANO-MESHED MICROCAPSULE

The porosity of the nano-meshed surface and the diameter of the microcapsules can be tuned by adjusting the ambient humidity during the electrospray process and the flow rate of the polymer solution respectively.

The porosity of the surface was sensitive to ambient humidity, and increased by electrospraying at higher humidity (Fig.5). This is presumably because the amount of the condensed water on the surface of the electrosprayed

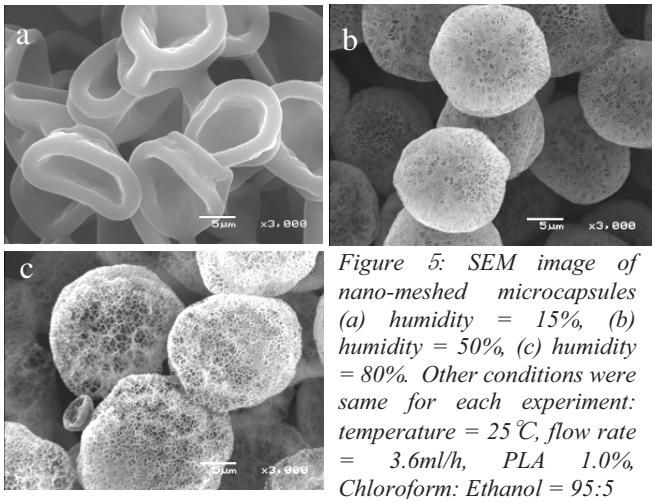

Figure 5: SEM image of nano-meshed microcapsules (a) humidity = 15%, (b) humidity = 50%, (c) humidity = 80%. Other conditions were same for each experiment: temperature = 25℃, flow rate = 3.6ml/h, PLA 1.0%, Chloroform: Ethanol = 95:5

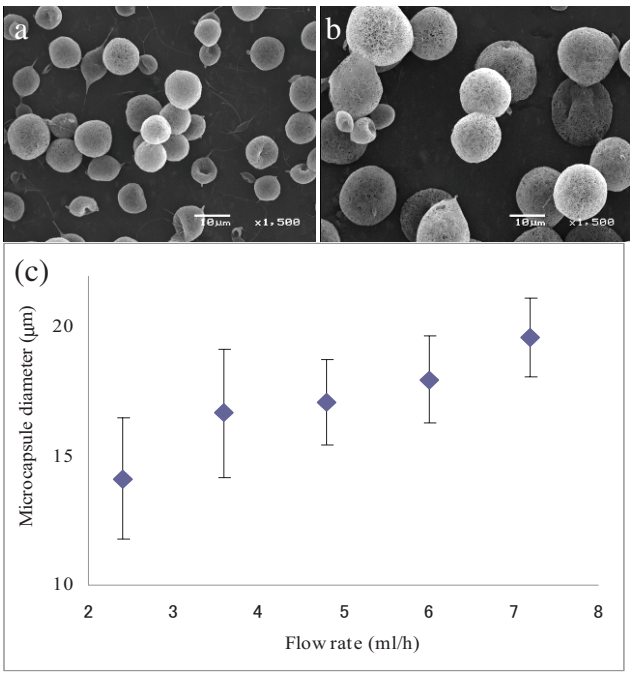

Figure 6: (a) SEM image of nano-meshed microcapsules: flow rate = 2.4ml/h, (b) SEM image of nano-meshed microcapsules: flow rate = 6.0ml/h, (c) Plot of microcapsule diameter as a function of the flow rate of the solution in the ejection nozzle. Experimental conditions except flow rate were same for each experiment: temperature = 25℃, humidity = 80%, PLA 1.0%, Chloroform: Ethanol = 95:5, Voltage = DC20kV

droplet of the polymer solution increases under high humidity, leaving larger pores on the final products through the formation of larger hydrophilic domain during phase separation process.

The diameter of the nano-meshed microcapsule was tunable from ~ϕ10μm to ϕ20μm by adjusting the flow rate of the solution to the nozzle. The diameter was increased with faster flow rates (Fig.6). A faster flow rate seems to cause the increase of the initial diameter of the electrosprayed jet of the solution from the nozzle, resulting in larger initial droplets formation by instability of the surface.

DIRECT SURFACE PATTERNING OF THE NANO-MESHED MICROCAPSULE

The nano-meshed microcapsules have the characteristics of both a nanofiber and a microparticle. That is, the surface of the microcapsule is composed of nanofibers ~φ100nm, and at the same time, can be treated as an individual particle. In this section we introduce a method using an electrostatic lens placed concentric to the ejection nozzle in order to focus the nano-meshed microcapsules. This set up can realize microcapsule formation and simultaneous direct patterning of the microcapsules on a substrate, whereas conventional electrospun nanofibers are difficult to be directly patterned due to its continuous form.

Schematic setup of the improvised electrospray and direct patterning system is shown in Fig.7a,b. All the process is done in a closed chamber capable of temperature and humidity control. The polymer solution is supplied to the nozzle from a motorized syringe, and the grounded target substrate is mounted onto a PC-controlled X-Y-Z stage. The electrosprayed nano-meshed microcapsules are focused at the bottom of the lens, and arbitrary shapes can be patterned in a single step by moving the stage (Fig.7c). In the following experiment, DC20kV was applied to ejection nozzle and DC15kV was applied to the electrostatic lens.

To verify the formation and simultaneous electrostatic focusing of the nano-meshed microcapsule, microcapsules were converged to a single spot on a glass substrate (Fig.8a). The height was increased depending on the electrospray duration, whereas the bottom width was kept constant due to electrostatic focusing (Fig.8b). The diameter of the pillar was ϕ~500μm at the bottom, and was decreased to ϕ~200μm in the upper part. By moving the X stage, a straight line composed of nano-meshed microcapsule was drawn on the glass substrate (Fig.8c). The depicted line was observed to be ~500μm in width. Using programmable motorized X-Y stage, arbitrary shape can be pattered on the substrate (Fig.8d). Contrary to the previously reported patterning methods of electrospun nanofibers by fabricating electrodes on the target substrate, the direct patterning method developed herein has high flexibility in modifying the pattern, which is suitable for fabricating customized products in medical applications.

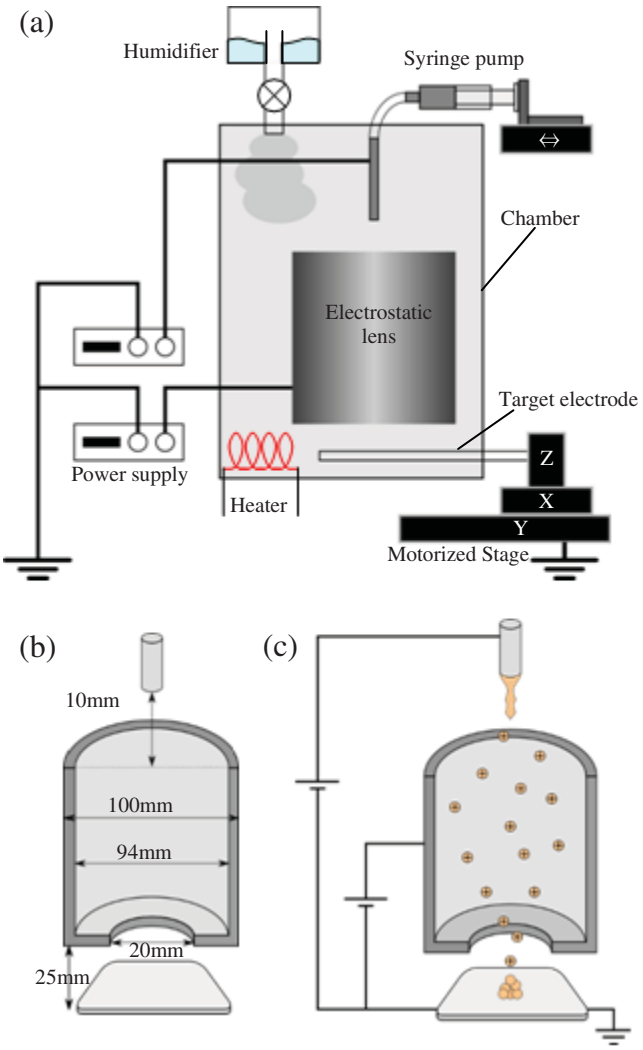

Figure 7: (a) Schematic of the improvised phase separation assisted electrospray patterning system (b) Dimensions of the setup with an electrostatic lens (c) Schematic of the formation and the focusing process of nano-meshed microcapsules using electrostatic lens.

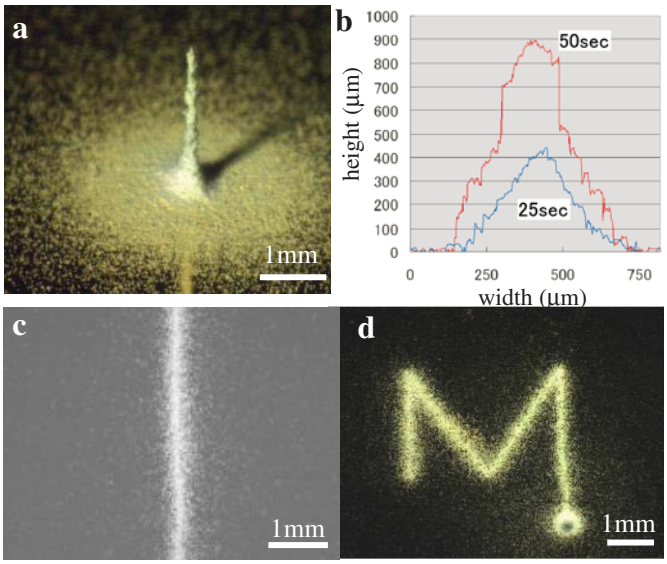

Figure 8: (a) Converged nano-meshed microcapsules using the electrostatic lens, (b) Cross-sectional profile of the deposited structure composed of microcapsules: The height was increased depending on the electrospray duration, whereas the bottom width was kept constant due to electrostatic focusing. (c) SEM image of a line drawn using the patterning system, (d) "M" shaped 2D pattern drawn using the patterning system as a demonstration

BIOCOMPATIBILITY OF THE NANO-MESHED MICROCAPSULE

In recent years, there has been an enormous rise in interest for biomedical research, having been one of the most beneficial fields owing to the advancement in nanotechnology. Among various biomedical researches, tissue engineering is advancing rapidly towards the realization of a totally new medicine. One of the challenges in tissue engineering is to design an ideal scaffold capable of both providing structural support and supplying nutrients for cells.

Electrospun nanofibers have been explored extensively as components of scaffolds because their large surface area and high porosity are suitable for cell adhesion and liquid circulation. However, there are still difficulties lying in the micro-patterning of electrospun nanofibers to create an arbitrary scaffold because of its continuous form.

One of the most attractive applications of the nano-meshed microcapsule is for usage as a component to create a scaffold, since it has fine nanofibrous surface like electrospun nanofibers, and at the same time, it can be patterned in an arbitrary shape as a particle.

To verify the application of the nanofibrous surface patterning method using nano-meshed microcapsules for tissue engineering, human hepatocyte cell was cultured on the nano-meshed microcapsules. Good cell adhesion and proliferation was observed after 48 hours of cultivation (Fig.9). It can be speculated that the topological characteristics of the nano-meshed microcapsules are favorable for cell adhesion and that the hollow structure of the capsules will allow nutrients and gases to circulate within the structures suitable for three-dimensional cell proliferation. Thus, the biocompatibility of the nano-meshed microcapsules was confirmed.

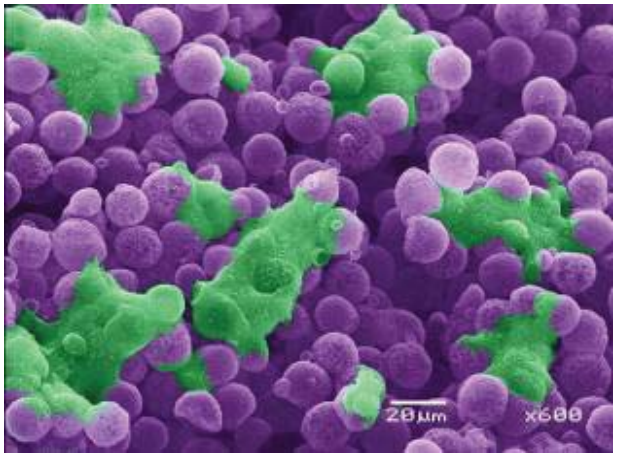

Figure 9: Pseudo-colored SEM image of human hepatocyte cells (green) cultured on the patterned nano-meshed microcapsules (purple) for 48h

CONCLUSIONS

This paper has presented a new morphology "nano-meshed microcapsule" created by phase separation assisted electrospray. The microcapsule has excellent uniformity in size and shape and possesses a nanofibrous surface which is ideal for cell culture. Using the microcapsules, nanofibrous surface patterning was realized by applying a secondary electric field created by an electrostatic lens. The patterning resolution was as high as 500μm. It was also confirmed that this surface patterning exhibited good cell adhesion indicating the possibility for further applications in biomedical and tissue engineering fileds.

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