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Enhancement of cell growth on honeycomb-structured polylactide surface using atmospheric-pressure plasma jet modification

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ABSTRACT

In this paper, we compare the cell growth results of NIH-3T3 and Neuro-2A cells over 72 h on flat and honeycomb structured PLA films without and with a two-step atmospheric-pressure nitrogen-based plasma jet treatment. We developed a fabrication system used for forming of a uniform honeycomb structure on PLA surface, which can produce two different pore sizes, 3–4 μm and 7–8 μm, of honeycomb pattern. We applied a previously developed nitrogen-based atmospheric-pressure dielectric barrier discharge (DBD) jet system to treat the PLA film without and with honeycomb structure. NIH-3T3 and a much smaller Neuro-2A cells were cultivated on the films under various surface conditions. The results show that the two-step plasma treatment in combination with a honeycomb structure can enhance cell growth on PLA film, should the cell size be not too smaller than the pore size of honeycomb structure, e.g., NIH-3T3. Otherwise, cell growth would be better on flat PLA film, e.g., Neuro-2A.

1. Introduction

Biomaterials have attracted much attention for the past two decades because of their potential biomedical applications on human body [1–3]. Good biomaterials should possess outstanding mechanical properties, surface properties, stability, biocompatibility, and easy and environmentally friendly fabrication process, to name a few [4–6]. With good biocompatibility, biomaterials should be non-cytotoxic, non-antigenetic, of uniform and high cell viability. These properties can induce cell migration and proliferation, as well as the synthesis of extracellular matrix components required for tissue growth [7,8]. How to fabricate biomaterials that meet the above criteria is important for their future biomedical applications.

Among various types of biomaterials, polylactic acid (PLA), the biodegradable polymer that is derived from renewable plant sources such as corn, becomes increasingly important in the field due to its stability in thermal and mechanical properties [9,10]. However, PLA is relatively hydrophobic with a static water contact angle of approximately 80°. Indeed, this leads to expected low cell affinity that can cause an inflammatory response from the living host, which could hinder extensive applications of PLA. The successful implementation of biomaterials relies on stable mechanical properties and controlled surface properties. The bioresponses to a polymer implant are mainly determined by surface properties, including surface functionality, energy (wettability), morphology and charge. Introducing different types of functional groups helps improve the surface properties that favor positive bio-compatibility in general.

Improving the biocompatibility of the material by introducing polar hydroxyl (—C—OH), carbonyl (—C=O), carboxyl —(C—O—O—H), oxygen containing functional groups and amino group ($-NH_2$) on the polymer surface not only increases the wettability and surface energy of the polymer surface, but also improves the early cell attachment and protein adsorption [8,11–13]. Cell attachment is one of the important factors of cells anchoring on the surface. It can affect activities of cells on the biomaterial surface, including cell proliferation and differentiation. The cell attachment is initialized with the formation of a cell adhesive protein layer from serum containing media, which can be enhanced by incorporation of functional groups onto the surface. The incorporation of amino groups may decrease the surface hydrophobicity and provide active sites that other biomolecules such as collagen, gelatin, or RGD peptides can be further immobilized, obtaining a surface on which cells can grow well [14,15].

In addition, porous polymer films have been studied for many years, because they can be used in catalysts, organic scaffolds, vascular scaffolds, cell culture substrate, cell recognition, and even in

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optical applications. Recently, a self-organized pattern that is called a honeycomb pattern (or breath figure pattern) was proposed in 1994 [16]. Some studies have shown that the organized honeycomb pattern possesses the ability to immobilize biomolecules or proteins. Hiroshi et al. [17] found that the honeycomb-structured film could prove cell adhesion and proliferation by protein adsorption via observation of fibronectin. Therefore, the advantages of this pattern on the biomaterial surface may improve the biocompatibility, cell attachment or proliferation so that it becomes one of the several ways to modify surface properties. The pore size plays a significant role during cell growth. Keiko et al. [18] observed that with different pore sizes of honeycomb, the cardiac myocytes behaved with obvious differences, such elongation and morphology of growth. By controlling the pore size of the honeycomb, we can affect the growth of the cell.

The mechanism of honeycomb pattern formation is of strong convection, in which the solvent evaporates quickly and the temperature on the surface decreases to −6–0 °C [19]. Water droplets in moist air condense and nucleate on the surface. The droplets sink into the surface by convection, form and organize on the surface into a highly ordered array if properly controlled. The formation of the pattern is also called breath figure [20]. The technique of forming an organized array based on the breath figure can be generally classified into the airflow technique, cold-stage casting technique, casting on water technique and emulsion technique. In the current study, we chose the airflow technique as our method to form and control the honeycomb pore size, which basic operational principle is described next. The polymer solution is cast in substrate and humid airflow blows on the solution surface to condense the water drops and evaporate the solvent. When the balance of water condensation and evaporation is achieved, the honeycomb structure is found on the surface. The factors, including properties of the polymer such as crystallinity and molecular weight, concentration and water addition to the solution, properties of solvent, relative humidity in the fabrication process, environmental temperature, coating substrate and forming time, can all greatly affect the uniformity and pore size of the honeycomb structure [20–24].

Biocompatibility is affected by the interactions between the biomaterial surface and host tissue, which heavily depends on the surface properties. The usual method is to fabricate biomaterials with adequate bulk properties followed by a special treatment to adapt the surface properties by functionalization of the material surface to control the biological response. Some reviews have highlighted plasma surface modification as an outstanding surface treatment technique owing to its non-line of sight, low temperature and dry processing. Low-pressure plasma surface modification on polymers has been intensively studied [25–27] in terms of physical and chemical properties for almost two decades. However, operation at reduced pressure increases the cost since one needs to employ very expensive vacuum equipment. On the other hand, several applications require atmospheric or continuous in-line processing, which further restrains the use of low-pressure discharges in these areas. Recently, atmospheric-pressure (AP) plasmas have been favored because of their distinct advantages compared to low-pressure plasmas. Atmospheric-pressure plasmas overcome the disadvantages of low-pressure plasmas [28,29]. Previously, we have shown the incorporation of amino groups using a two-step nitrogen DBD-APPJ treatment procedure promoted cell attachment and proliferation on a flat PLA film [30]. We have applied this 2-step plasma treatment for fast incorporation of amino functional groups using the post-discharge jet region. It was carried out in two steps, which include nitrogen APPJ with 0.1% oxygen addition and nitrogen APPJ with 5% ammonia addition in sequence.

In this study, we apply this two-step DBD-APPJ treatment procedure to modify the surface chemical properties of flat and honeycombed PLAs. We optimize the effects of concentration, water addition and processing time to form a regular honeycomb pattern of PLA. We intend to combine the advantages of plasma treatments to incorporate amino functional groups and the outstanding features of a honeycomb-patterned PLA surface to produce a highly biocompatible material at a very low cost and in an efficient manner.

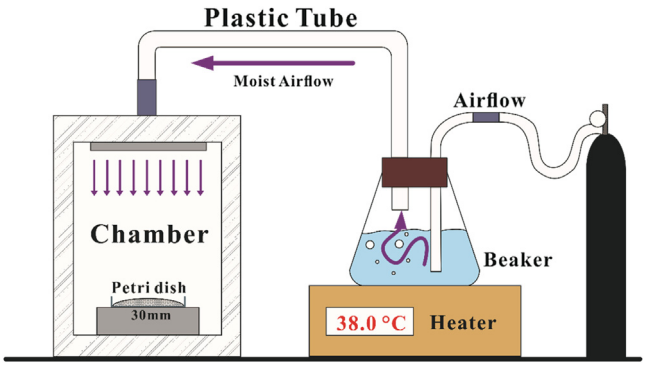

Fig. 1. Schematic diagram of honeycomb formation system.

2. Experimental method

2.1. Honeycomb fabrication system

Fig. 1 shows the schematic diagram of the honeycomb fabrication system which consists of an air gas bottle, a beaker filled with deionized water with a heater underneath to control the water temperature, and a chamber to create a controllable humid space. The air flow from the gas bottle was fixed at 3 slm, passed through the water-containing beaker in order to carry water vapor into the chamber. By increasing the power into the heater, we could increase water temperature and generate more water vapor. The water inside the beaker was fixed at 38 °C. When the system was stable, the chamber was maintained at 35 °C and 99.9% relative humidity, which could be a suitable environment for honeycomb structure formation. Eventually we placed the PLA solution that was uniformly coated on Petri dishes (diameter 30 ± 1 mm, height 5.5 ± 0.5 mm) into the chamber for the forming process.

We had performed numerous experiments to test which polymer could be well coupled with our system; we found the 719935-Poly (D,L-lactide) (Mw: 10–18 K, 0.16–0.24 dL/g, Sigma-Aldrich) to be the most appropriate PLA type for forming the honeycomb structure matching to our facility design. Two kinds of solvent was used to dissolve PLA from powder to liquid; one was chloroform (J. T. Baker, 9180), while the other was tetrahydrofuran (THF: Macron, 8498-08). The PLA was dissolved at room temperature, stirred for 3 h, then casted on the clean glass Petri dish with different conditions. When the solvent was completely evaporated, the patterned films were formed.

2.2. Plasma modification

For plasma modification of the PLA surface, we have applied a previously constructed DBD-APPJ system which was built for surface modification [30], as shown in Fig. 2. This system consisted of two parallel copper electrodes (50 × 50 × 8 mm each) with embedded cooling water. Each electrode was covered with a ceramic plate (70 × 70 × 2 mm). The dielectric plates were extruded 5 mm from the end of the electrodes (in the flow direction), which prevented the electrode assembly from arcing. The distance between the two dielectric plates (ceramic) was kept as 1 mm throughout the study. The assembly of electrodes and dielectrics was then covered by an insulation layer made of Teflon to provide safety and