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Two-dimensional amine-functionality gradient by plasma polymerization

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ABSTRACT

A two-dimensional (2D) gradient was created by employing two-step diffusion-controlled plasma polymerization of allylamine on polypropylene membrane, with varied deposition durations composed of two stages. The wettability gradient was examined by water contact angle measurements which clearly demonstrated that the gradient was well manipulated by varying the treatment duration, thus controlled the surface coverage and resulted in the average water contact angle ranged from 110° to 25°. On the created 2D gradients, the cell responses toward the distribution of nitrogen content were imaged by confocal laser microscopy. Moreover, a correlation based on experimental data revealed a linear relationship between the nitrogen content and water contact angle. The addition of serum proteins assisted also the adhesion and growth of L-929 cells. The methodology in fabricating the 2D gradient demonstrated the flexibility of the plasma technique which can be further used to create different types of well-defined gradients for applications in surface functionalization, biomedical devices, and material screening.

1. Introduction

Surface gradients contain continuous changes of physicochemical characteristics such as wettability and functionalities along the sample length [1]. For the past few decades, various gradients have been created for the purposes of screening newly developed materials [2], improving biochemical assays [3], and investigating cellular interactions [4–6]. Surfaces possessed gradients exhibit particular advantages such as high-throughput, efficient in time and consumables usages, and less operational error for samples prepared in different batches for the applications in nanotechnology and biomaterials [7–11].

Two different approaches were commonly employed to create wettability gradients: surface coatings and surface modifications. The surface coating by immobilization of self-assembled monolayers (SAMs) allowed the incorporations of surface functionalities effectively with specificity [12–15]. However, extensive washing steps and longer experimental duration were generally required for preparing gradients by chemical methods. Alternatively, the surface modification processes by applying high energy sources (plasmas [16–18], corona discharges [3,19,20], and plasma polymerizations [6,21,22]) to create gradients allow the incorporations of chemical functionalities efficiently on different types of substrates in a solvent free manner.

Amine and amide functionalized surfaces via plasma polymerization which were proved to promote cell adhesion and growth provided versatile applications in sensor fabrications [23–25] and for the biocompatibility improvement of biomedical devices [26,27]. Different research groups have reported that the growth of human dermal fibroblasts was promoted to more than 250% and 150% by plasma polymerizations using different precursors [28–30]. For the applications in medical devices, Yang et al. demonstrated that allylamine plasma polymer coated 316L stainless steel possessed excellent stability for the immobilization of heparin on cardiovascular stents [31]. The creation of gradients by applying plasma polymers were illustrated in various applications using different precursors [32]. Although the deposition of allylamine plasma polymers was studied by different research groups [6,21,22,32–34], the creation of allylamine gradient by plasma polymerization was not yet reported.

In the developments of polymer chemistry and physics, the combinatorial chemistry was successfully applied for high throughput research on the creation of novel functional polymers [35,36]. Recently, this advantageous method was adapted for preparing two-dimensional gradients to study the responses of living cells on biomaterials [37,38]. This particular gradient demonstrated the possibility to optimize the coverage of PHEMA via controlling the concentration of fibrinogen to study the growth of MC3T3-E1 osteoblast cells [39]. An alternative two-dimensional (2D) radial gradient of wettability and dodecyltrichlorosilane (DDS) content was prepared to investigate the adhesion of alga spores. The spores tended to favor the position on the 2D gradient where exhibited the

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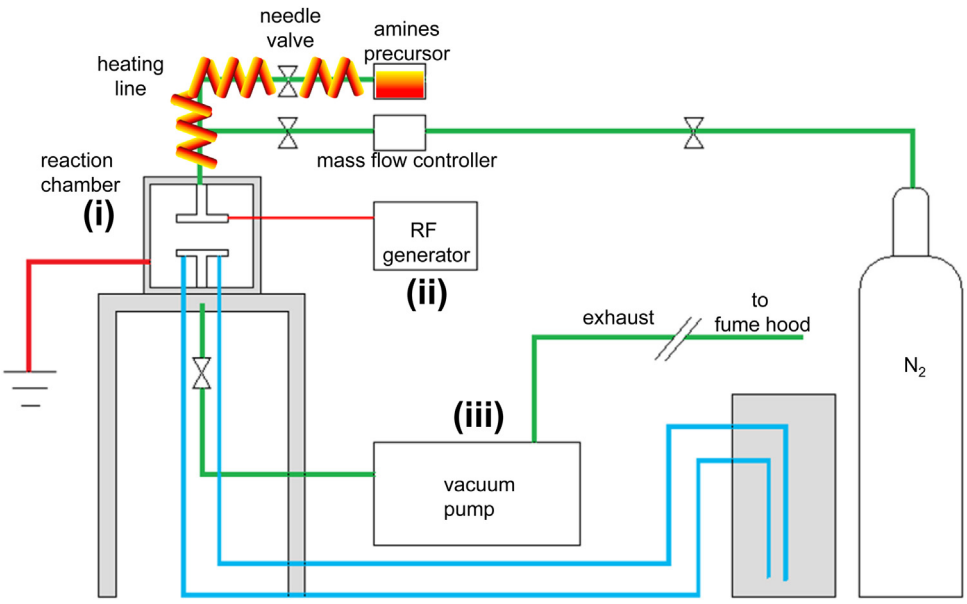

Fig. 1. Plasma polymerization apparatus which is composed of (i) reaction chamber, (ii) RF generator, and (iii) pumping system.

most hydrophilic and the least DDS concentration [40]. Moreover, the combination between chemical gradient of poly(D,L-lactide) and poly(ε-caprolactone) (PDLA/PCL) with the annealing temperature of the copolymers provided the optimized parameters for MC3T3-E1 osteoblasts [41].

In this study, an allylamine 2D gradient was fabricated by plasma polymerization on the polypropylene sample with 1 cm × 1 cm surface area. Each sample was deposited twice with the duration composed of 10 min (*A*) and 30 min (*B*). The prepared 2D allylamine gradient was not reported previously although research on combinatorial chemistry has been carried out and reported [35–38]. In order to illustrate the usability of the created 2D gradient as a screening tool, L-929 fibroblast cells were cultivated on the gradient and the cell responses were examined by both confocal laser scanning microscopy and lactate dehydrogenase assay.

From the experimental results, it was found that the initial stage of plasma polymerization played the most important role on dictating the cell growth on the 2D gradient. The content of serum proteins was also an important factor that promoted the cell proliferation in comparison with the less significant parameter, the duration of the second plasma deposition. The created 2D gradient containing amine functionality, evaluated by cell responses and simulation results, provides an alternative method to evaluate the usefulness for newly developed materials which assist to accelerate the examining procedures for the properties of materials. Therefore, the proposed technique can be used further for the developments and applications in functional devices and biomaterials.

2. Experimental

In this study, 3 factors were employed to design the experiments: plasma deposition time *A* and *B* (representing 10 and 30 min), and two replicates for each sample. The responses of live cells were evaluated by cultivation of L-929 fibroblasts with varied serum addition *C* (by 0% and 10% fetal bovine serum). The composition of the deposition durations resulted in four representative 2D gradient samples: $A_{10}B_{10}$, $A_{10}B_{30}$, $A_{30}B_{10}$, and $A_{30}B_{30}$, where the subscripted values represented the plasma deposition time *A* and *B*.

2.1. Plasma polymerization of allylamine

Polypropylene membrane (PP, Celgard®, Hoechst) was cut to 1 cm × 1 cm, attached onto cover slip glass, and washed extensively by immersing in ethanol and deionized water prior to the utilization. The plasma polymerization was conducted in a plasma reactor, modified from previous work (Fig. 1) [42]. In brief, the system is consisted of three main parts: (i) a reaction chamber; (ii) a radio-frequency generator; and (iii) a vacuum system. In the plasma reactor, a stainless steel anode with 2 cm thickness was placed at the center of the vacuum chamber, and located 10 cm above pumping outlet. A shower-type cathode was separated with the anode by 7 cm, both electrodes were circular with diameter of 15 cm. Allylamine (Alfa Aesar, 98%) was utilized as received without further purification, and was plasma polymerized under total pressure of 100 mTorr, flow rate of 10 sccm, and preheating at 40 °C, with deposition time combined of 10 and 30 min. A mask was applied to cover the PP substrate, where the gap between the mask and substrate was 1 mm which therefore allowed the entry of limited reactive species into the mask and reacted with the substrate.

The procedure for preparing the 2D gradient is illustrated in Fig. S1 (in supplementary information). The sample was placed inside plasma chamber under the mask, and exposed to plasma for the first deposition time *A* (10 or 30 min). Next, the sample was rotated by 90°, and undergone the second deposition with time period *B* (10 or 30 min). The corner that was exposed twice under the plasma deposition was marked by an asterisk.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bej.2013.02.022.

2.2. Cell culture

The L-929 fibroblast cells were cultivated in a humidified incubator with temperature 37 °C and 5% CO_2 control. All culture media were purchased from Sigma: Dulbecco's modified eagle medium (DMEM-high glucose) (56439C); trypsin, lyophilized powder (T4799); EDTA (E6758); fetal bovine serum (FBS, F2442); sodium bicarbonate (S5761); sodium pyruvate (P5280); and L-glutamine (G8540). The initial seeding cell density was 20,000 cells/mL and the cultivation time was set for 48 h (surpassing the doubling time