Fabrication of Ordered Arrays of Biodegradable Polymer Pincushions Using Self-Organized Honeycomb-Patterned Films

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Summary: Nano- and micropatterned structures of tissue engineering scaffolds made of biodegradable and biocompatible polymers profoundly influence cell behavior. The present study describes a technically simple and inexpensive method to rapidly fabricate hexagonal arrays of biodegradable polymer pillars (pincushions). As precursors to these polymer pincushion arrays, highly regular porous biodegradable polymer films (self-organized honeycomb-patterned films, called honeycomb films) were prepared on a glass substrate using a simple casting technique. Scanning electron microscope observations revealed that the honeycomb film was composed of a top and bottom layer. This double-layered structure is attributable to the self-organization of hexagonally packed arrays of water droplets that form the template. When we peeled off the top layer of the honeycomb film under ambient conditions using adhesive tape, we obtained arrays of polymer pincushions on both side of the glass substrate and on the adhesive tape. Each air hole is surrounded by six pincushions, each with a diameter of 0.1-1 μm. We also studied factors that determine the morphology of the pincushions, such as the thermal and mechanical properties of the polymers used. It was shown that the heights, widths, and distances of separation between the pincushions could be controlled by the choice of polymer and the pore structure of the original honeycomb film. Such well-ordered, biologically inspired pincushion structures could find application in biomedical, photonic, and electronic materials.

Keywords: pillar; pincushion; scaffold; self-organization; tissue engineering

Introduction

Three-dimensional (3D) scaffolds fabricated from biodegradable polymers play critical roles in tissue engineering as temporary extracellular matrices and have therefore been widely used.[1] Biocompatible 3D scaffolds of appropriate architectures facilitate cell adhesion, proliferation, differentiation, and eventual tissue regeneration in a natural manner. Cultures of cells on 3D biodegradable scaffolds may create tissues suitable either for applications in reconstructive surgery or that can serve as novel in vitro model systems. It is well-known that nano/micro patterns fabricated on the surfaces of such scaffolds significantly influence the morphology, proliferation, differentiation, and function of various cells.[2,3]

Direct-write lithography is widely used in research because it is inexpensive and offers flexibility in pattern generation. Currently, electron-beam lithography is

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the most commonly used direct-write technique. Further, a number of highly useful fabrication methods, including phase separation,[4–6] porogen leaching,[7] gas foaming,[7] fiber meshing,[8] supercritical fluid processing,[9] microsphere sintering,[10] and three-dimensional printing[11] and soft lithography techniques,[12] have been developed and used to fabricate tissue engineering scaffolds from biodegradable polymers. Each of the abovementioned techniques is unique and requires specific process conditions. However, these fabrication techniques require a large amount of energy and involve numerous steps. In addition, the starting materials used in these techniques have limited availability.

As a result, there is a high demand for suitable alternative methods that can be used to prepare scaffolds from functional polymers.[13,14] Biology is full of examples of so-called bottom-up fabrication—self-organization of organic and inorganic components into hierarchical, sophisticated structures under mild ambient conditions. For example, established interference patterns found on butterfly wings show the same self-cleaning properties as do lotus leaves and photonic crystals.[15] Gecko feet, research on which has been a major topic of interest, have nearly 500,000 keratinous hairs or setae and can generate a strong adhesive force.[16,17] Inspired by the organization exhibited in biological structures, we expected the self-organization of biodegradable polymers into hierarchical and sophisticated structures to be a suitable alternative to the conventional techniques of nano/micro fabrication of tissue engineering scaffolds. Self-fabrication of polymers requires physiological conditions, in contrast to conventional nano/microfabrication techniques, which require harsh conditions.[13,14] The self-organization technique can be used with various biodegradable polymers because of its physical generality. Regular structures have been formed during the casting of polymer solutions on solid surfaces; for example, self-organized honeycomb-patterned films (honeycomb films) with highly regular porous structures can be prepared under humid casting conditions.[18–22] We have reported the formation of pincushion structures on a glass substrate after peeling off the top layer of the nonbiodegradable honeycomb films using adhesive tape.[23] However, nonbiodegradable scaffolds are not ideal for biomedical applications such as tissue engineering and drug delivery. For such applications, it would be advantageous to create nano- and micropatterned structures that can gradually degrade and be resorbed by the body.

The present study describes the fabrication of hexagonal arrays of biodegradable polymer pincushions to be used as novel tissue engineering scaffolds. As a precursor to these biodegradable polymer pincushions arrays, highly regular honeycomb films were prepared on the glass substrate by a simple casting technique using essential biodegradable, and biocompatible polymers such as poly (ε-caprolactone) (PCL), poly(L-lactide) (PLA), poly(D,L-lactide-co-glycolide) (PLGA), and poly(3-hydroxybutyrate) (PHB). This fast and inexpensive method of fabricating biodegradable polymer pincushions can be used to produce nano- and microtopographies for cell-support scaffolds for tissue engineering, drug delivery, and use as tissue adhesives for medical applications.

Experimental Part

Materials

Biocompatible and biodegradable polymers used for the fabrication of pincushion arrays were PCL (M_w of 70,000–100,000, Wako), PLA (M_w of 85,000–160,000, Sigma), PLGA (M_w of 40,000–75,000, Sigma), and PHB (M_w of 100,000, Sigma). Polystyrene (PS, M_w of 200,000, Aldrich), poly(methyl methacrylate) (PMMA, M_w of 35,000, Aldrich), Poly(bisphenol A carbonate) (PC, M_w of 28,000, Aldrich), and poly(tetrafurfuryl methacrylate) (PTHFMA, M_w of 240,000, Scientific Polymer Products) were used as nonbiodegradable reference polymers. A copolymer of N-dodecylacrylamide and

ω-carboxyhexylacrylamide (abbreviated as Cap in this paper) was used as an emulsifier. It was synthesized by a method reported previously.[22] It is amphiphilic because it forms a stable monolayer at the air-water interface. The molecular weight of Cap, estimated by size-exclusion chromatography, was 4.5×10^4 g/mol. Water was purified using a Millipore system (Milli-Q, Millipore). Organic solvents and other chemicals used were commercially available and were used as received, without further purification.

Preparation of Honeycomb Films and Pincushions

Honeycomb films were fabricated from biodegradable and nonbiodegradable polymers and Cap on a glass substrate by employing a previously described method.[22] In brief, the polymers and Cap (10:1 wt%) were dissolved in chloroform at a concentration of 4.0 g/L. The polymer solution was poured onto the glass substrate under a blast of highly humid air 1.0–3.0 L/min, 25 °C, relative humidity: 70–80%). The cast volume of the polymer solution ranged from 2.5 to 10 mL. After complete evaporation of the solvent and condensed water, the surface structure was observed under an optical microscope (BH-2, Olympus, Japan). The honeycomb film was peeled off of the substrate at room temperature.[23] The top surface of the honeycomb film formed on the glass substrate was peeled off using adhesive tape (Nichiban, Japan) (Figure 1). Tilted and cross-sectional structures of the film were observed using a scanning electron microscope (SEM, S-3500N, Hitachi, Japan). The static contact angles of the films were measured at room temperature (25 °C) by the sessile drop method using a contact-angle goniometer (Face CA-A, Kyowa Interface Science, Japan).

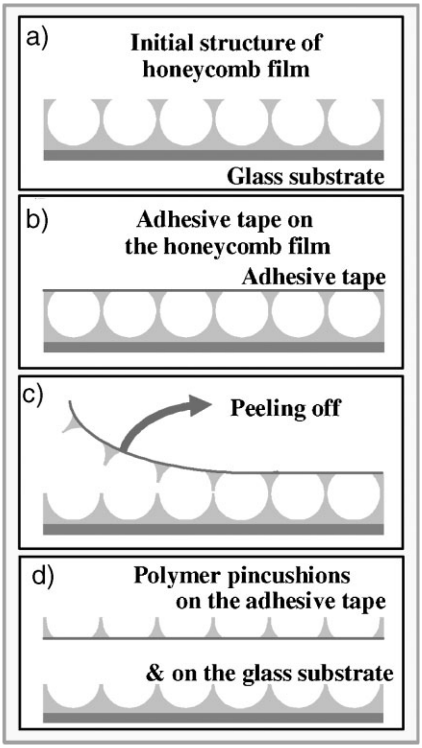

Figure 1.
Schematic representation of fabrication procedure of polymer pincushions (a) Cross sectional view of an initial honeycomb film on a glass substrate. (b) Stick an adhesive tape on the honeycomb film. (c) The top layer of the honeycomb film was peeled off by the adhesive tape. (d) Arrays of polymer pincushions on both side of the glass substrate and on the adhesive tape.

Results and Discussion

Biological polymers such as collagen and hyaluronic acid can be used in tissue engineering because they promote cell adhesion and chemotactic responses. However, the immunogenicity, potential risk of disease transmission, sourcing and poor handling, and weak mechanical properties of these polymers are topics of concern. The mechanical properties and biodegradability of tissue engineering scaffolds should resemble those of healthy tissues during tissue regeneration. In this study, four synthetic biodegradable and biocompatible polymers— PCL, PLA, PLGA, and PHB— were investigated that offer versatile options for such use as scaffolds. The use of these polymers in certain clinical practices has been approved by the U.S. Food and Drug Administration; hence, they can be used to fabricate honeycomb films by a self-organization technique. A procedure for fabricating biodegradable polymer pincushions has been developed; this method requires neither high temperatures nor harsh chemical conditions, and can therefore be used with a variety of polymers. Pincushions based on some commonly used polymers, including PS, PC, PMMA, and PTHFMA, were fabricated as controls. The honeycomb films of PS, PMMA, PC, PTHFMA, PCL, PLA, PLGA, and PHB, which are precursors to the respective polymer pincushions, were observed using scanning electron microscopy (SEM). The micrographs of various polymers in Figure 2 show a highly regular hexagonal pore arrangement (honeycomb-patterned structure) as well as a uniform pore structure (pore size: approximately 5–7 μm). Although all of the abovementioned films were prepared under the same casting conditions, there exist slight differences in morphology due to the solubility of the polymers in chloroform and the viscoscity, surface tension, and density of the solutions. These parameters affect the shape of the water droplets used as a template for the honeycomb film. A schematic and a real cross-section of the regular honeycomb film are shown in Figure 3.

Using an SEM, we observed that the honeycomb film had a top layer and a bottom layer and laterally interconnected structures with side pores. The tilted and side-view images of the honeycomb film reveal two hexagonal lattices connected at the vertices of the hexagons by vertical columns. This double-layered structure reflects the morphology of the template, which is a self-organized and hexagonally packed array of water droplets. The use of a water-immiscible solvent for preparing the polymer solution resulted in condensation of water droplets after the solution was cast onto the substrate; this was due to the evaporative cooling of humid air used in the experiment. Self-packed, monodispersed water droplets that formed on the surface of the polymer solution acted as a temporary template for the pores. In general, the condensed water droplets are not stable and eventually start to coalesce. In order to prepare a highly regular honeycomb film, it is necessary to stabilize the water droplets. The amphiphilic copolymer Cap prevents the fusion of the water droplets during pattern formation. Cap acts as a surfactant and contributes to the stabilization of the water droplets at the interface of the polymer solution and water. As a result, fusion of the water droplets is prevented by the intervening amphiphilic polymer layer. Most polymers dissolved in a water-immiscible solvent can thus be made to form honeycomb films through the addition of amphiphilic compounds. Various experimental factors affect the structure of the pores. A uniform pore size can be achieved

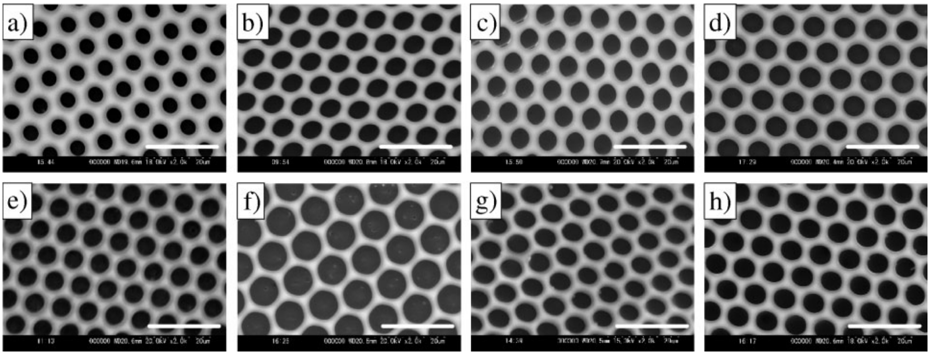

Figure 2.
Scanning electron micrographs of the original structures of the honeycomb films made of (a) PS, (b) PMMA, (c) PC, (d) PTHFMA, (e) PCL, (f) PLA, (g) PLGA, and (h) PHB (Bar: 20 μm)

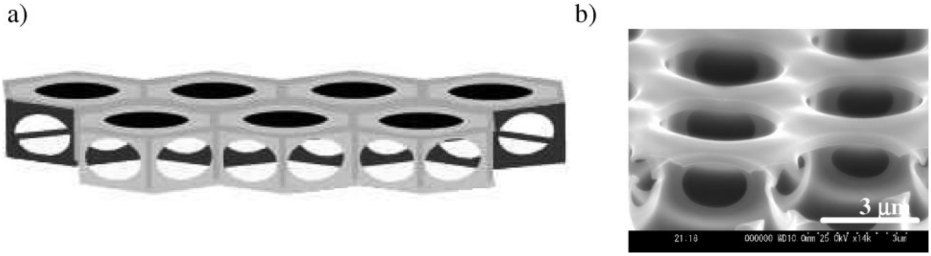

Figure 3.
(a) Schematic and (b) real cross-sections of the regular honeycomb film.

by changing the casting conditions.[22] The casting volume controls the pore size of the fabricated honeycomb films. The rims of the films widened as the pore size increased. The porosity of each film was found to be approximately 50%.

When the top layer of the honeycomb film was peeled off using adhesive tape under ambient conditions, arrays of polymer pincushions were formed on both sides of the glass substrate and on the adhesive tape. Figure 4a shows a complete image of the pincushions and typical SEM images of the pincushion structures (Figure 4b) on both sides of the glass substrate and (Figure 4c) on the adhesive tape. Each air hole is surrounded by six pincushions with a diameter of approximately 0.1–0.5 μm. Figure 5 shows the peeling Interface of the honeycomb film. For reference, only half of the honeycomb film was formed into a pincushion pattern. The contact angle of the typical pincushion was approximately 153°, regardless of the pore size, indicating that its hydrophobicity is greater than that of the honeycomb film, whose contact angle was 107°. Figure 6 shows the tilt-angle SEM images of the pincushion structures obtained from various honeycomb films. The morphologies of these pincushions, which were prepared under the same

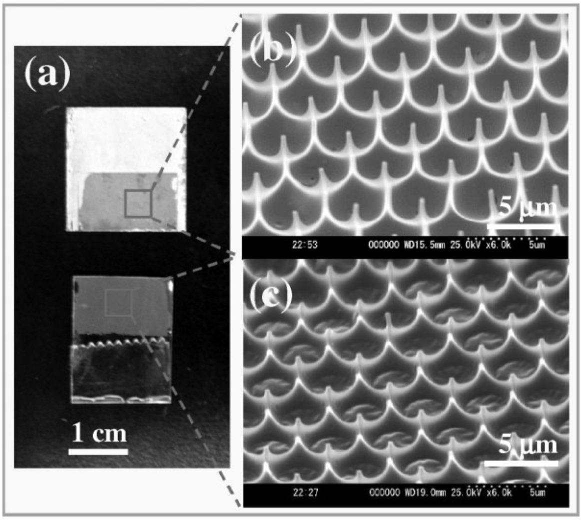

Figure 4.
(a) Image of PS pincushions on both sides of the glass substrate and on the adhesive tape. Tilt-angle (55°) scanning electron micrographs of the surface topography of arrays of PS pincushion arrays formed on (b) the glass surface and (c) adhesive tape.

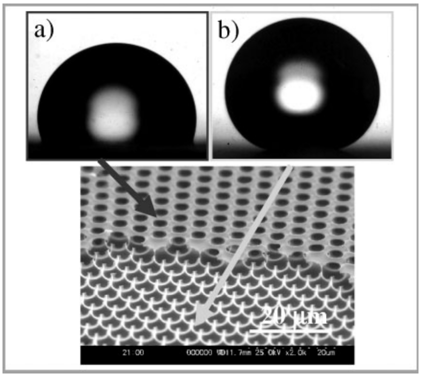

Figure 5.
Profile of a water droplet on (a) a honeycomb film and (b) a polymer pincushion surface formed from PTFHMA. Only half of the honeycomb film has been formed into a pincushion pattern.

peeling conditions, can be categorized into two types: i.e. vertically aligned (Figures 6a–d) and hairly aligned (Figures 6e–h). The SEM images reveal that the pincushions obtained from PS, PC, PMMA, and PTHFMA were vertically aligned. However, pincushions obtained from PCL, PLA, PLGA, and PHB were both vertically and tilted hairly aligned. These pincushions were approximately 0.1–1 μm in diameter. The spacing between the pincushions was approximately 7–10 μm. The pincushions had uniform diameters and were evenly distributed on both sides of the glass substrate and adhesive tape with a periodicity of 0.5–5 μm. The film remaining after the top layer of the honeycomb film had been peeled off was able to be removed from the glass substrate as a free-standing film. In contrast, the pincushions formed on the tape were found to adhere strongly to the tape and were flexible. The heights, widths, and distances of separation of the pincushions were dependent on the type of polymer used and the pore size of the original honeycomb film.

As shown in Figure 6e, PCL pincushions showed an elongated hair-like morphology compared to other polymer pincushions, including the nonbiodegradable polymer controls. We also studied factors that determine the structure of the pincushions, such as the thermal and mechanical properties of the polymers used. A comparative study on the physicomechanical properties of several biodegradable polyesters has been performed.[24] The study determined the thermal and tensile properties and the flexural storage modulus of the polyesters as a function of temperature. The thermal and mechanical properties of the typical biodegradable and nonbiodegradable polymers tested in that study are listed in Table 1.[24]

PCL is an aliphatic polyester and is semicrystalline with a low glass transition temperature (Tg) of approximately −60 °C and a low melting point (Tm) of approximately 60 °C; hence, it is always in a

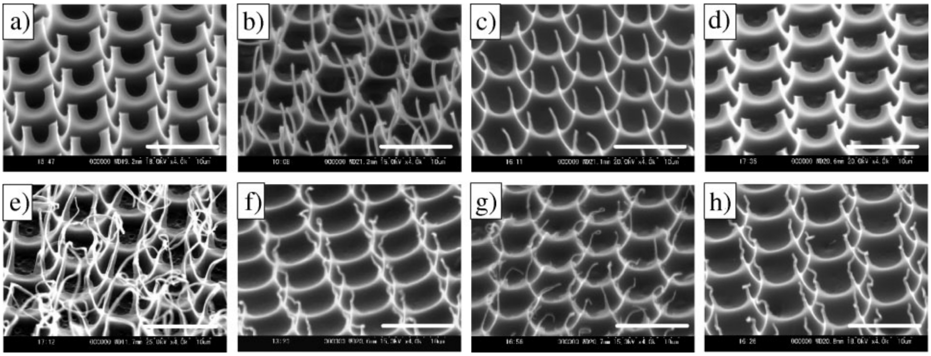

Figure 6.
Tilt-angle scanning electron micrographs of the polymer pincushions (a) PS, (b) PMMA, (c) PC, (d) PTHFMA, (e) PCL, (f) PLA, (g) PLGA, and (h) PHB (Bar: 10 μm).

Table 1.
Thermal and mechanical properties of typical biodegradable and nonbiodegradable polymers.

Polymer	Abbreviation	Tg (°C)	Tm (°C)	Tensile strength (MPa)	Tensile modulus (GPa)	Elongation to break (%)
Poly(ε-caprolactone)	PCL	−62	57	16	0.4	80
Poly(L-lactide)	PLA	54–59	170–178	28–50	1.2–3	2–6
Poly(D,L-lactide-co-glycolide) (50/50)	PLGA	45–50	–	50	2.4	2.6
Poly(3-hydroxybutyrate)	PHB	1	171	36	2.5	2.5
Poly(methyl methacrylate)	PMMA	115	–	55–76	2.4–3.1	2–5
Poly(ε-caprolactone)	PCL	−62	57	16	0.4	80
Poly(L-lactide)	PLA	54–59	170–178	28–50	1.2–3	2–6

rubbery state at room temperature. Another interesting property of PCL is that it has a low tensile modulus of approximately 0.4 GPa and a high elongation to break of approximately 80%. Though PLA has a Tg of 55 °C, it is semicrystalline with a high Tm of approximately 170 °C and is harder than PCL. PLGA is the most widely used biodegradable noncrystalline polymer, with a Tg of approximately 45 °C. PHB is a crystalline polymer produced by microorganisms that melts at approximately 170 °C, as does PLA. PLA, PLGA, and PHB have elongations to break of approximately 3%, which are considerably lower than the elongation to break of PCL. PMMA, PS, and PC have higher Tg values than do the four biodegradable polymers investigated. PC has an elongation to break of 150% and high impact strength. Both PMMA and PS have moderate toughness. PCL was selected as a biodegradable polymer, because it is more pliable than PLA, PLGA, or PHB. The low Tg and tensile modulus of PCL may affect the elongated hair-like morphology of the PCL pincushions. Among the parameters, the lower Tg of PCL could be a key parameter determining the elongated hairy morphology, although other factors in Table 1 and peeling conditions such as the molecular weight and polydispersity as well as the crystallinity of the polymers, and fabrication temperature are also involved. In fact, we found that the vertically aligned PCL pincushions were obtained, as were those of other polymers, by peeling at temperatures below Tg that were reached using liquid N_2. This indicates that both sharp and hairly pincushion structures could be controlled by peeling off temperature at above or below Tg.

To the best of our knowledge, this is the first report of a simple method to fabricate highly organized and hairly elongated pillars from biodegradable polymers using self-organized honeycomb films as precursors. The fabrication is performed under physiological conditions, in contrast to conventional industrial processing techniques that require harsh conditions; this makes our fabrication method simpler and less expensive than conventional techniques. As mentioned previously, micro- and nanostructured surfaces of scaffold materials have a marked influence on cell adhesion. These pincushion surfaces may improve cell adhesion and positively affect the flow of nutrients and wastes. In particular, biologically inspired hairy pincushions made from PCL may generate significant adhesive force. The reason for this expectation is that these structures are similar to those found on the feet of geckos.[16,17] This study makes significant progress in understanding cell–material interactions.

Conclusions

Using a self-organized honeycomb structure as a precursor, we have successfully fabricated biodegradable polymer pincushions with hexagonally arranged pillars by peeling off the top layer of the obtained films using adhesive tape. The desired morphology of the pincushion structures, including their heights, widths, and distances of separation, can be obtained by choosing the appropriate polymer, (Tg), and the pore structure of the original honeycomb film. The fabrication method proposed in this work is a novel and simple method to prepare biodegradable polymer pincushions that avoids the use of conventional lithography. Unlike other template or lithographic methods, the advantage of this method is the ease and low energy cost with which it allows patterned surfaces to be fabricated using a variety of materials; further, it is inexpensive and easily scalable. We believe that the method presented in this work will aid the pursuit of new and exciting opportunities in microfluidics, chemical microreactors, biochemical detection, thermal management of microelectronics, bio-optics, implants, and many areas beyond tissue engineering scaffold.[23]

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