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Cell patterning on polylactic acid through surface-tethered oligonucleotides

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ABSTRACT

Polylactic acid (PLA) is a candidate material to prepare scaffolds for 3-D tissue regeneration. However, cells do not adhere or proliferate well on the surface of PLA because it is hydrophobic. We report a simple and rapid method for inducing cell adhesion to PLA through DNA hybridization. Single-stranded DNA (ssDNA) conjugated to poly(ethylene glycol) (PEG) and to a terminal phospholipid (ssDNA-PEG-lipid) was used for cell surface modification. Through DNA hybridization, modified cells were able to attach to PLA surfaces modified with complementary sequence (ssDNA′). Different cell types can be attached to PLA fibers and films in a spatially controlled manner by using ssDNAs with different sequences. In addition, they proliferate well in a culture medium supplemented with fetal bovine serum. The coexisting modes of cell adhesion through DNA hybridization and natural cytoskeletal adhesion machinery revealed no serious effects on cell growth. The combination of a 3-D scaffold made of PLA and cell immobilization on the PLA scaffold through DNA hybridization will be useful for the preparation of 3-D tissue and organs.

1. Introduction

Regenerative medicine and tissue engineering have attracted much attention as effective methods for treating various diseases. Transplantation of oligodendrocyte progenitor cells derived from embryonic stem cells has been clinically examined to treat spinal cord injury [1]. There are plans to treat Parkinson's disease and Type 1 diabetes through the transplantation of appropriate cells [2,3]. Cell sheets have been applied to regeneration of the epidermis, mucous membrane and corneal epithelium [4–6]. Techniques to regenerate the three-dimensional (3-D) structure of living tissue and organs composed of several different cell types are desired to extend the horizon of regenerative medicine [7–9]. The 3-D printer has attracted much attention as a promising tool to prepare 3-D tissue and organs. Commercially available 3-D printers employ polylactic acid (PLA) owing to its low melting temperature. In addition, PLA is biodegradable, and thus has been used clinically to repair various simple tissues, such as connective tissue [10], bone [11] and dura mater [12]. Therefore, PLA is a suitable candidate material from which to prepare 3-D tissue or organs using a 3-D printer. Although a 3-D printer can make a scaffold out of PLA that mimics natural tissue structure, there are other difficulties. Cells do not adhere or proliferate well on PLA surfaces because of its hydrophobicity [13,14]. It is also difficult to arrange different cell types in their desired positions.

Previously, we reported a simple and rapid cell patterning method whereby single-stranded DNA (ssDNA) conjugated to poly(ethylene glycol) (PEG) and a terminal phospholipid (ssDNA-PEG-lipid) was used to modify cells [15,16]. The cells were then able to attach to surfaces that had been modified with complementary sequence (ssDNA′) through DNA hybridization. In this study, we developed a method to immobilize cells on the PLA surface in a spatially controlled manner using the described DNA hybridization method. Sheets and fibers were prepared from PLA, including PLA carrying terminal carboxylic acid, through which PLA films and fibers were modified with ssDNA′. Cells treated with ssDNA-PEG-lipid were applied to the PLA surface carrying ssDNA′; through complementary DNA hybridization, the treated cells became attached at the position at which the ssDNA′ was immobilized (Fig. 1a).

2. Materials and methods

2.1. Materials

Poly(D,L-lactic acid) carrying terminal carboxylic acid (PDLLA-COOH; MW ⩾ 16 kDa), and poly(L-lactic acid) (PLLA; MW ⩾ 130 kDa, terminal group: alkyl ester) were purchased from DURECT Co. (Cupertino, CA, USA). α-*N*-Hydroxysuccinimidyl-ω-maleimidyl

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poly(ethylene glycol) (NHS-PEG-Mal, MW: 5000) and 1,2-dipalmitoyl-sn-glycerol-3-phosphatidylethanolamine (DPPE) were purchased from NOF Corporation (Tokyo, Japan). Benzene, 1,4-dioxane, 2-propanol, dichloromethane, triethylamine, chloroform, diethyl ether, trypsin, penicillin–streptomycin mixed solution and Blocking One were purchased from Nacalai Tesque (Kyoto, Japan). Minimal essential medium (MEM), RPMI 1640, fetal bovine serum (FBS), CellTracker™ Green CMFDA and CellTracker™ Orange CMRA, AlexaFluor 488-conjugated anti-mouse IgG, and AlexaFluor 594-conjugated phalloidin were purchased from Invitrogen Co. (Carlsbad, CA, USA). Anti-vinculin antibody (ab18058) was purchased from Abcam (Cambridge, UK). Dulbecco's phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) was purchased from Nissui Pharmaceutical Co. Ltd. (Tokyo, Japan). 4-(4-*N*-Maleimidophenyl)butyric acid hydrazide–HCl (MPBH) was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). ssDNA-SH sequences designated as SeqA, SeqA′, SeqB, and SeqB′ and listed in Table 1 (received as protected forms for thiol by 6-mercapto-1-hexanol), and SeqA carrying fluorescein isothiocyanate (FITC) at the 5′-end (FITC-SeqA) were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride (EDC) and Hoechst 33342 were purchased from Dojindo (Kumamoto, Japan).

2.2. Immobilization of ssDNA on PLA film (ssDNA-PLA film)

PLA film was formed on cover slips (24 mm × 24 mm × 0.17 mm) by spin-coating with 1% PDLLA-COOH/PLLA mixed solution (weight ratio: 1/1) in 1,4-dioxane at 3000 rpm for 60 s using a spin-coater (1H-DX, Mikasa, Tokyo, Japan). The PLA spin-coated surface was incubated at 110 °C for 30 min, and immersed in a solution of EDC/MPBH (EDC/MPBH = 1, 1 mg ml^{-1} in DMSO) for 12 h at room temperature (RT; 25 ± 3 °C) to introduce maleimide groups onto the PLA surface (Mal-PLA film). The glass plates were washed thoroughly with pure water and 2-propanol, sequentially. ssDNA-SH was obtained by removing a protection group for thiol (6-mercapto-1-hexanol) according to the manufacturer's instructions. Mal-PLA films were immersed in a solution of ssDNA-SH (500 μg ml^{-1} in PBS) and incubated for 1 h at RT and then rinsed with PBS to remove unimmobilized ssDNA from the surface (ssDNA-PLA film).

2.3. Characterization of ssDNA-PLA film surfaces

During each modification step, the film surfaces were characterized by water contact angle measurement and X-ray photoelectron spectroscopy (XPS). Contact angles on each substrate were determined by the sessile drop method using a contact angle meter (CA-X, Kyowa Interface Science Co. Ltd., Saitama, Japan); the averages of four individual measurements were obtained. Atomic compositions of surfaces were determined using an XPS meter (ESCA-850V, Shimadzu Co., Kyoto, Japan). A magnesium target was used as an X-ray source with an electric current from a filament (30 mA, 8 kV). The takeoff angle of the sample surface was 15°, and the base pressure of the analysis chamber was $<1 \times 10^{-5}$ Pa. Sweeps were conducted five times with 0.1 eV steps.

Table 1
Nucleic acid sequences of oligoDNA.

ssDNA and ssDNA′	Nucleic acid sequences
SeqA	5′-HS-AAAAAAAAAAAAAAAAAAAA-3′
SeqA′	5′-HS-TTTTTTTTTTTTTTTTTT-3′
SeqB	5′-HS-TGCGGATAACAATTTCACACA-3′
SeqB′	5′-HS-TGTGTGAAATTGTTATCCGCA-3′

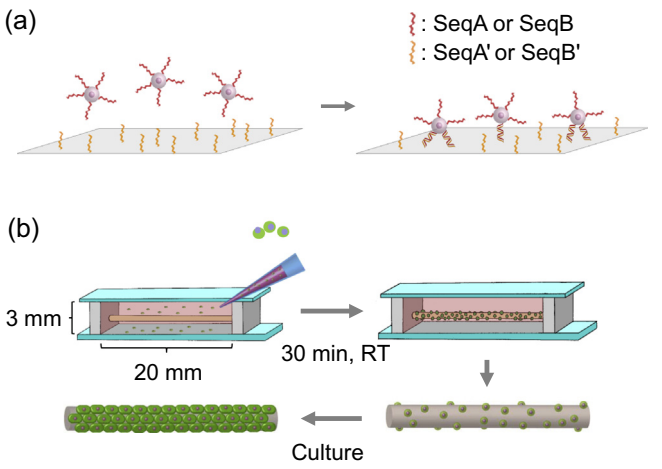

Fig. 1. A schematic diagram of cell attachment to PLA fibers through DNA hybridization. (a) Cell attachment to a PLA fiber. Cells modified with ssDNA-PEG-DPPE are applied to the ssDNA′-PLA fiber and incubated to allow for specific binding of ssDNAs. SeqA′ and SeqB′ indicate base sequences complementary to SeqA and SeqB, respectively. (b) Culturing ssDNA-presenting cells on the ssDNA′-PLA fiber. An ssDNA′-PLA fiber was placed such that it was floating between two glass plates. A cell suspension was applied to the gap formed by the two glass plates and incubated for 30 min. After washing, the fiber was incubated in culture medium supplemented with 10% FBS to allow for proliferation of the cells on the fiber.

2.4. Immobilization of ssDNA on PLA fiber (ssDNA-PLA fiber)

PLA fiber was prepared using a wet spinning method according to Ref. [17] with slight modifications. A mixed solution of 10% PDLLA-COOH/PLLA (weight ratio: 1/1) in 1,4-dioxane was extruded into a water coagulation bath through a 21-G needle at 50 μl min^{-1} using a syringe pump (PHD 2000; Harvard Apparatus, Holliston, MA, USA) and the fiber was drawn by hand. The diameter of the fiber was 100 ± 50 μm. Each fiber was stored in a desiccator for 1 h, and incubated at 110 °C for 30 min. The fibers were immersed in a solution of EDC/MPBH (EDC/MPBH = 1, 1 mg ml^{-1} in DMSO) for 1 day at RT to introduce maleimide groups onto the fiber surface (Mal-PLA fiber). The Mal-PLA fibers were washed thoroughly with pure water and 2-propanol, sequentially; next, they were immersed in a solution of ssDNA-SH (500 μg ml^{-1}, 80 μM in PBS) and incubated for 1 h at RT to immobilize ssDNA on the surface (ssDNA-PLA fiber) (Scheme 1).

2.5. Binding of complementary DNA to ssDNA-PLA fiber

The ssDNA-conjugated PLA fiber was incubated with 500 μg ml^{-1} of FITC-ssDNA' (whose ssDNA sequence was complementary to that of the PLA fiber-conjugated ssDNA) in PBS solution for 30 min at RT. The fiber was rinsed with PBS, and then observed under a fluorescence microscope (IX71, Olympus, Tokyo, Japan). For comparison, ssDNA-PLA fiber, Mal-PLA fiber and PLA fiber were also examined.

2.6. Synthesis of ssDNA-PEG-DPPE

We synthesized ssDNA-PEG-DPPE following a previously reported method [15]. Briefly, DPPE (25 mg), Mal-PEG-NHS (200 mg) and triethylamine (100 μL) were mixed in 5 ml dichloromethane and stirred for 72 h at RT. The mixture was poured into ice-chilled diethyl ether to precipitate Mal-PEG-DPPE. After suction filtration, Mal-PEG-DPPE was dissolved into benzene and freeze-dried. ssDNA-SH was obtained by removing a protection group for thiol from ssDNA according to the manufacturer's instructions. Mal-PEG-DPPE (375 μg ml^{-1}) and ssDNA-SH (75 μg ml^{-1}, 12 μM)