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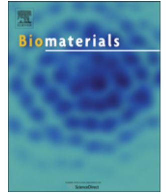

The significance of hydrated surface molecular mobility in the control of the morphology of adhering fibroblasts

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ABSTRACT

The effects of the hydrated molecular mobility and the surface free energy of polymer surfaces on fibronectin adsorption and fibroblast adhesion were investigated. ABA-type block copolymers composed of polyrotaxane (PRX) with different number of threaded α-cyclodextrin (α-CD), random copolymers with similar chemical composition to the PRX block copolymers, and conventional polymers were prepared to determine a wide range of hydrated molecular mobility (Mf) values estimated by quartz crystal microbalance-dissipation (QCM-D) measurements. Fibronectin adsorption was highly dependent on surface free energy, and high surface fibronectin density resulted in a large projected cell area on the polymer surfaces. However, the morphology of adhering fibroblasts was not explained by the surface free energy, but it was found to be strongly dependent on the Mf values of the polymer surfaces in aqueous media. These results emphasize the importance of Mf in the discussion of the elongated morphology of adhering fibroblasts on various polymer surfaces.

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1. Introduction

An understanding of the critical factors affecting cellular responses on the surface of materials is important when designing functional biomaterials. When artificial materials are placed in a biological environment, the primary protein–material interaction that occurs is significant protein adsorption [1]. The protein molecules that are adsorbed on the artificial material then continuously migrate over the surface until they determine the thermodynamic standpoint for final conformations. Conformational changes in the bioactive domains of adsorbed protein molecules resulting from their exposure are the cause of many biological responses such as cell adhesion, activation, and immune reactions [2–5]. In particular, it has been clearly shown that the magnitude of a cellular response, such as adhesion density, proliferation rate, or projected cell area, is directly related to the adsorption density of protein molecules on material surfaces [6,7].

While the relationship between the adsorption density of protein molecules and the number of adhering cells has been clarified, the critical factors that affect the adhesion states of cells have not been broadly studied. Recently, several papers have reported the critical factors relating to the adhesion states of various cells. For instance, the stiffness of materials is known to be an important factor in regulating the expression of various protein tyrosine kinases in adhering fibroblasts, leading to different adhering morphologies [8]. This material–cell communication provides important information for designing a cell culture platform that regulates proliferation rate or lineage of stem cell differentiations [9–11]. Although the factors that affect cell adhesion behavior have been studied, some aspects of the topic remain unexplored.

Because cells communicate with the external environment in a dynamic manner, the molecular mobility of polymer surfaces is considered to be one of the important factors that dominate cell adhesion [12]. However, difficulties in modulating molecular mobility and characterizing the mobile nature of polymer surfaces preclude the study of the relationship between molecular mobility and cellular responses. Previously, we reported that the dynamic nature of polymer surfaces can affect the adhesion morphology of fibroblasts in the presence of serum [13]. The degree of hydrated molecular mobility (Mf) was determined by measuring energy dissipation against micro-vibrations transmitted to the polymer surfaces in an aqueous environment by using quartz crystal

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microbalance-dissipation (QCM-D) equipment. It was confirmed that fibroblasts on the highly dynamic polymer surfaces (high Mf) developed by a molecularly mobile polyrotaxane (PRX) segment showed an elongated morphology (low aspect ratio), whereas less mobile random copolymer surfaces showed round-shaped morphology, which suggests that molecular mobility is one of the critical factors dominating the fate of fibroblasts. However, more discussion on Mf versus adhesion morphology is required for a broad range of polymer surfaces to generalize the effect of Mf on cell adhesion behavior. Furthermore, the effect of Mf on biological responses, compared to a traditional interfacial parameter such as surface free energy, should also be discussed. To this end, the present study was designed to clarify the Mf-adhesion morphology relationship of fibroblasts on polymer surfaces with a wide range Mf values, as well as a variation of surface free energy, by using two series of PRX block copolymers, random copolymers, and conventional polymers. The adsorption tendency of cell-adhesive fibronectin and the consequent adhesion of fibroblasts was considered in terms of surface free energy and Mf. Because the adhering morphology of fibroblasts is also affected by various biomolecules such as growth factors in serum [14], the present study was conducted in a simplified system, namely serum-free, fibronectin precoated polymer surfaces.

2. Materials and methods

2.1. Materials

2-Methacryloyloxyethyl phosphorylcholine (MPC) was obtained from NOF Co. (Tokyo, Japan), and α-cyclodextrin (α-CD), *n*-butyl methacrylate (BMA), sodium hydride, iodomethane, α,α′-azobisisobutyronitrile (AIBN), and all the organic solvents were purchased from Tokyo Kasei Co. (Tokyo, Japan) and used as received. Methyl methacrylate (MMA), 2-methoxyethyl methacrylate (MEA), *iso*-butyl methacrylate (iBMA), and 2-hydroxyethyl methacrylate (HEMA) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA) and were distilled by passing them through a basic alumina column to remove an inhibitor prior to use. Polyethylene glycol (PEG) (average molecular weight of 20,000) (PEG 20k) was also purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA), and the PRX block copolymers and random copolymers were synthesized and used as we have reported previously [13,15].

Goat polyclonal antibody to mouse immunoglobulin G (IgG) conjugated with horseradish peroxidase (HRP) was purchased from Abcam Inc. (Cambridge, MA, USA), and fibronectin from human serum and mouse monoclonal anti-fibronectin antibody (clone FN-15) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Hoechst 33258 was purchased from Dojindo Lab (Kumamoto, Japan), Alexa Fluor 546 Phalloidin and other biological reagents were purchased from Gibco Invitrogen Corp. (Grand Island, NY, USA), and NIH3T3 fibroblasts were from Riken Cell Bank (Japan).

2.2. Synthetic process of conventional polymer samples

Conventional polymer samples (PBMA, PiBMA, PMEA, PMMA, PHEMA) were synthesized in the following manner. Each monomer was dissolved in 5 mL of 1 M toluene/ethanol (50/50) mixed solvent. To this, 4.3 mg of AIBN was added, and the mixture was bubbled with dry Ar for 10 min. The mixture was then sealed and placed in an oil bath at 70 °C for 24 h. The reaction mixture was precipitated in cold diethyl ether (PHEMA) or methanol (PBMA, PiBMA, PBMA, and PMMA). The excess solvent was then removed by evaporation, and the precipitates were once again thoroughly stirred with the solvent. The resulting precipitants were then dried in vacuo at 40 °C for 24 h.

2.3. Surface characteristics

The synthesized copolymers (5 mg) were initially dispersed in 5 mL ethanol. Next, 5 mL of water was added to prepare 0.05 wt% of clear polymer solution. In the

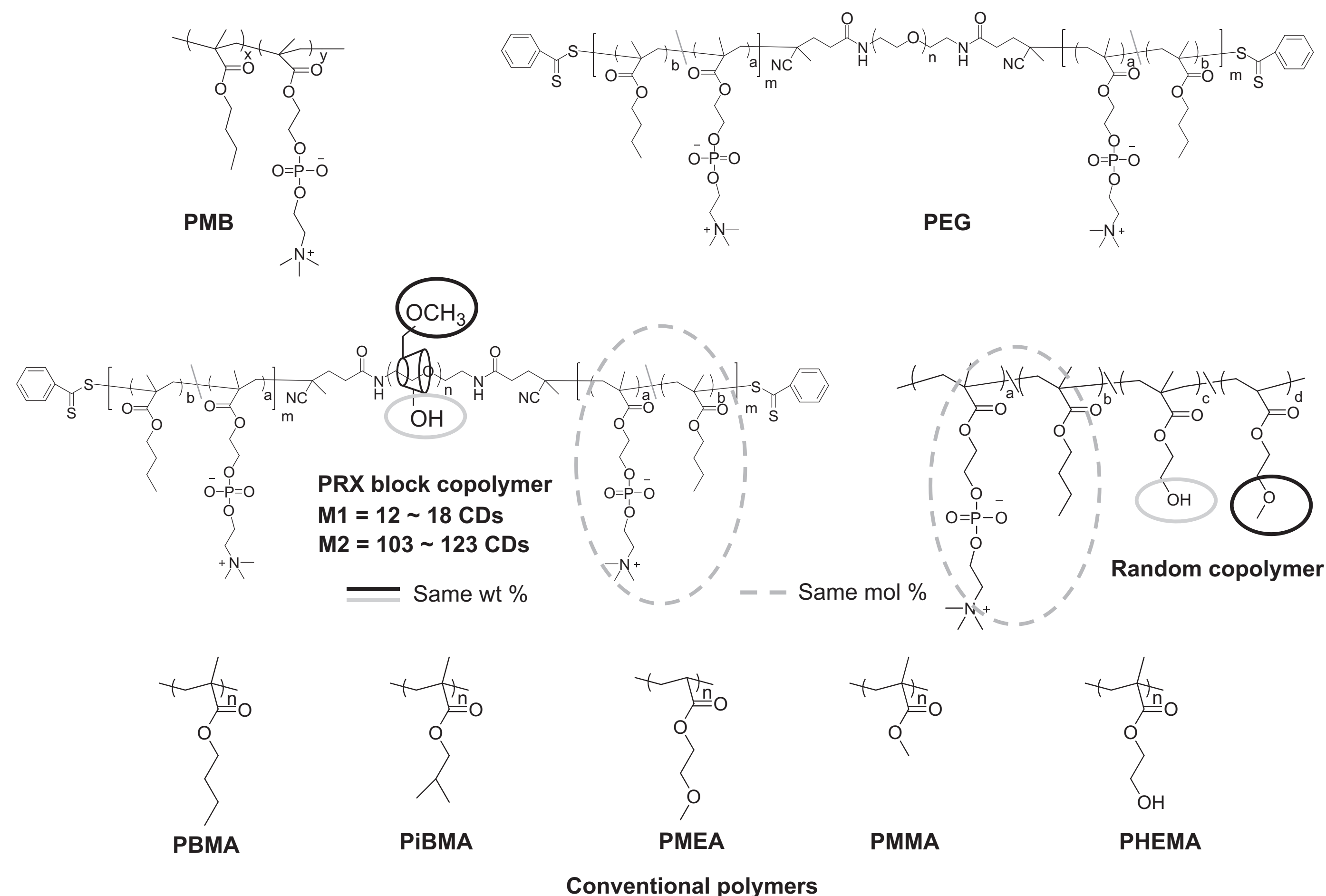

Fig. 1. Molecular structure of polymer samples.

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