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Selective adsorption of L1210 leukemia cells/human leukocytes on micropatterned surfaces prepared from polystyrene/polypropylene-polyethylene blends

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ABSTRACT

The objective of this study is to prepare polymeric surfaces which will adsorb L1210 leukemia cells selectively more than that of healthy human leukocytes in order to develop new treatment options for people with leukemia. Chemically heterogeneous and micropatterned surfaces were formed on round glass slides by dip coating with accompanying phase-separation process where only commercial polymers were used. Surface properties were determined by using optical microscopy, 3D profilometry, SEM and measuring contact angles. Polymer, solvent/nonsolvent types, blend composition and temperature were found to be effective in controlling the dimensions of surface microislands. MTT tests were applied for cell viability performance of these surfaces. Polystyrene/polyethylene-polypropylene blend surfaces were found to show considerable positive selectivity to L1210 leukemia cells where L1210/healthy leukocytes adsorption ratio approached to 9-fold in vitro. Effects of wettability, surface free energy, microisland size geometry on the adsorption performances of L1210/leukocytes pairs are discussed.

1. Introduction

Cell–polymer surface interactions which are important for disease treatment or design of biomedical products are not well understood at present. These interactions were related to five parameters such as type and percent distribution of the chemical groups on the surface [1], surface roughness [1–5], surface wettability [6–8], surface free energy (SFE) of the substrate [6,9], and size and shape of the cell used for testing [10,11]. The distance between surface patterns and focal adhesion clusters on the cell membrane is also an important parameter for the cell to form a strong adhesion on a substrate [1–3,12,13]. Shapes, functions and viability of the cells may change as a result of their interaction with a patterned substrate [14]. Chemical composition and morphology of a substrate are important parameters affecting the adsorption of proteins present on the cell surface and initial cell adhesion [15–17].

When blood contacts with a substrate, the adhesion properties of normal blood cells (i.e. leukocytes, platelets) change depending on the surface properties of the substrate [18,19]. The substrate is quickly covered with proteins present in the serum. Differences in topography, chemical composition, charge and wettability in the substrate surface result in changes in type, conformation and amount of the adsorbed proteins. Protein adsorption is a dynamic process and proteins do not stay on the surface permanently, and over time the higher-affinity proteins replace the previously pre-adsorbed lower-affinity proteins [20]. Protein adsorption increases with the increase in hydrophobicity of the substrate since water molecules in the serum interact weakly with the surface enabling more proteins to adsorb. On the other hand, protein adsorption also increases with the increase in the surface roughness due to the increase of the total surface area. At the end, composition and conformation of proteins are different on the surface depending on the hydrophobicity and roughness of the surface [20]. These variations in proteins are recognized by distinct cell adhesion molecules; hence, cell adhesion is highly dependent on cell type. Every cell has a different combination of adhesion molecules expressed on their surface. For example, leukocytes are well endowed with integrin adhesion receptors [21]. Moreover, cancer cells are known to alter their adhesion molecules in order to invade and metastasize [22–25]. Therefore, it will be possible to allow a desired cell type to attach to a substrate by changing surface topography, composition, and wettability. Our aim is to design a polymer surface which allows L1210 adhesion and inhibit healthy leukocyte attachment. This selectivity is expected to be a result of the fact that surface receptors of healthy leukocytes and L1210 cancer cells might be different.

L1210 mouse lymphocytic leukemia cells are one of the model cell types used in cancer studies. The adhesion properties of L1210

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leukemia cells to solids have been examined previously and it was found that they do not adhere to glass whereas they easily bind to embryonic fibroblast monolayers [26]. It was also determined that adhesion of L1210 cells increased linearly with the increase of the sulfo group (−SO_3H) concentration on sulfonated PS surfaces [27]. Adhesion properties of human leukocytes to surfaces were also investigated previously and it was found that the number of leukocytes which adhered to the polymeric surfaces modified by surface sulfonation or addition of other functional groups was higher than the non-modified surfaces [28]. The activation of adherent leukocytes was found to be dependent on surface topography, pattern geometry and surface chemistry of the substrate [18]. Davidson et al. showed that interaction of the normal cells with the topographically patterned surfaces was different from that of the cancer cells and plasma membranes of the normal cells were not easily deformed when the contact area between surface and cells was increased [4]. Yan et al. suggested that cell binding locations and properties can be controlled by designing specific micropattern sizes [29]. These findings show that micropatterned surfaces may be used in selective adsorption of different cell types.

Polymeric materials are widely used as biomedical materials. Surface patterning of polymers has been performed by applying several approaches such as lithography, UV light sensible photoresist, and use of specially synthesized polymers [30]. Most of these patterning methods are time consuming and expensive; however, Erbil et al. have demonstrated that a cheap commercial polymer such as polypropylene can be converted to a superhydrophobic surface by using solvent–nonsolvent phase separation process and this method can also be applied to large area surfaces and three-dimensional materials [31]. Polymer blends which are the physical mixtures of the independent polymers can preferably be used for this purpose because blending may provide optimization of the desired properties of the final surface [32]. Solvent evaporation rate, phase separation, solvent diffusion, and SFE of the polymers have substantial effects on surface morphology of the polymer blends [31,33–35]. Micropatterned surfaces can also be prepared using polymer blends [36,37]. Kajiyama and co-workers observed that PMMA formed cylindrical micropatterns with controllable diameters by creating crests on the PS structures using spin-coating with PMMA/PS blends [38]. Overney et al. obtained micropatterned surfaces by performing annealing process through formation of PS monolayers by spin-coating on poly-ethylene-co-propylene film [39].

Whitesides and co-workers were the first to use micropatterned surfaces in the biomedical field in 1997 [12] and determined that human cells die on solids with particular patterns whereas they remain alive on solids with different patterns and concluded that cell size and shape are important factors for this cellular behavior [2,12]. The interactions between microstructured surfaces and cells have been intensively investigated in the last decades and it was found that cells strongly respond to topographic changes on surfaces [40–42]. The amount and/or orientation of the initial protein adsorption on topographically reconstructed surfaces occur differently from the flat surfaces [41].

In this study, we prepared flat, rough and micropatterned surfaces on round glass slides using commercial polymers such as polystyrene (PS), high density polyethylene (HDPE), polyethylene-polypropylene copolymer (PPPE), ethylene-vinyl acetate copolymer (EVA), and polyvinyl alcohol (PVOH) and also polymer blends (PS/HDPE, PS/PPPE) by dip coating method. We determined the specific surfaces where selective adsorption of L1210 leukemia cells occurs but human leukocytes do not adsorb. These surfaces may be used during blood exchange transfusion of leukemia patients to remove the cancerous cells (like L1210 leukemia cell) out of blood. The effectiveness of the parameters such as surface roughness, surface chemical groups and wettability onto surface/cell selective adsorption was also studied. Smooth polymer surfaces were prepared to observe the effect of roughness. It was found that selective adsorption performance changed depending on the diameters of short cylindrical islands and separation distance between the islands on the polymer surfaces especially when PS/PPPE polymer blend surfaces were used where island diameters can be easily controlled.

2. Materials and methods

2.1. Materials

PVOH (Merck, M_w = 160,000), PS (Sigma–Aldrich, M_w = 350,000), HDPE (Basell Inc., HOSTALEN-GM8255, M_w > 1,000,000), PPPE copolymer containing 12% PE content by weight (Dow Chemical Co., VERSIFY 2300), and EVA copolymer containing 12% vinyl acetate content by weight (Dupont, ELVAX 660) were used as received. These polymers were dissolved in THF (Merck), xylene and toluene solvents (Tekkim, Turkey) to prepare polymer solutions. Ethanol (EtOH, Merck) was used as non-solvent. Ultrapure water, diodomethane, formamide, α-bromonaphthalene, and ethylene glycol (all from Merck) were used as contact angle drop liquids. Round glass slides (Thermo Scientific) with diameters of 13 and 15 mm were used as substrates. All biological materials used in this study are analytical grade and were purchased from Invitrogen and Sigma–Aldrich.

2.2. Preparation of homo- and copolymer films

PVOH was dissolved in water, PS and EVA in toluene, HDPE in xylene, PPPE in THF solvents at a constant concentration of 10 mg/mL. EtOH was added to PS and PPPE polymer solutions prepared in THF solvent by 10% (v/v). The withdrawal rate of the mechanical dipper was varied between 320–764 mm/min at room temperature for PVOH and PS polymers, at 60 °C for PPPE and EVA, and at 115 °C for HDPE. Clean round glass slides was kept in the polymer solution for 1 min to reach thermal equilibrium, and then withdrawn with a constant speed. Polymer films were kept in a desiccator for 3–4 h and completely dried in a vacuum oven at 40 °C overnight. PVOH film was dried under vacuum overnight, then was kept in glutaraldehyde solution for 2 h and then washed using distilled water and dried under vacuum at 50 °C overnight to prepare cross-linked PVOH surfaces. Surface sulfonation was carried out by submerging PS samples in sulfuric acid (60 vol.%) for 24 h. Sulfonated PS surfaces (PS-sulfo) were rinsed in deionized water, and air-dried.

2.3. Preparation of PS/HDPE and PS/PPPE blend films

PS, HDPE and PPPE polymer stock solutions were prepared in xylene and THF solvents at 10 mg/mL concentration at 10 °C below the boiling points of the solvents. PS/HDPE and PS/PPPE blend solutions were prepared by mixing the different compositions for a final concentration of 10 mg/mL and stirred mechanically for 2–3 h at 60 °C to reach equilibrium when THF/xylene mixture was used and 115 °C when only xylene was used. EtOH was added dropwise into the blend solution. Dip coating of the round glass slides by blend polymers was carried out with a mechanical dipper at a removal speed of 320–784 mm/min. Coated polymer films on glass slides were kept in a desiccator for 3–4 h and completely dried in a vacuum oven at 40 °C overnight. Thicknesses of the coatings were between 0.5 and 2.0 μm. Surface topography of rough polymer blends was examined by 3D profilometry (Nikon, Eclipse-LV100D Microscope) and environmental scanning electron microscopy (ESEM, Quanta 200 FEG).