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Surface energy effects on osteoblast spatial growth and mineralization

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Abstract

While short-term surface energy effects on cell adhesion are relatively well known, little is revealed as regards its later stage effects on cell behavior. We examined surface energy effects on osteoblastic cell growth and mineralization by using human fetal osteoblastic (hFOB) cells cultured on plasma-treated quartz (contact angle, $\theta = 0°$) and octadecyltrichlorosilane (OTS)-treated quartz ($\theta = 113°$). hFOB cells formed a homogeneous cell layer on plasma-treated quartz, while those cultured on OTS-treated quartz produced randomly distributed clump-like structures that were filled with cells (confirmed by confocal microscopy). Mineral deposition by hFOB cells was spatially homogeneous when cultured on hydrophilic surfaces. Furthermore, cells on hydrophilic surfaces exhibited increased mineralized area as well as enhanced mineral-to-matrix ratio (assessed by Fourier transform infrared spectroscopy), relative to cells on hydrophobic surfaces. Experiments using other types of osteoblast-like cells (MC3T3-E1, MG63, and SAOS-2) revealed more or less similar effects in spatial growth morphology. It was concluded that hydrophilic surfaces induce homogeneous spatial osteoblastic cell growth and mineral deposition and enhance the quantity (e.g., area) and quality (e.g., mineral-to-matrix ratio) of mineralization relative to hydrophobic surfaces. Our data suggest that surface energy effects on osteoblastic cell differentiation, especially mineralization, may be correlated with surface energy dependent changes in spatial cell growth.

Keywords: Surface energy; Osteoblast; Hydrophilic; Hydrophobic; Mineralization

1. Introduction

Signals from biomaterial chemistry, surface energy, charge, topography, wettability, and chemical/topographic patterning have the potential to affect bone forming cell behavior [1,2]. Short-term effects of these parameters have been investigated extensively and many studies, including ours [3–8], have demonstrated that biomaterial surface characteristics systematically affect osteoblastic cell adhesion, integrin expression and focal adhesion, cell signaling, spreading, cell number doubling, etc. However, relatively little has been revealed as regards later stage effects of these parameters on cell behavior.

Surface energy or wettability of biomaterials is of significant interest in biomedical applications involving cell–biomaterial interactions as surface wetting phenomena significantly affect various biological events at the sub-cellular and cellular level (protein adsorption, cell attachment and spreading, etc.). As a measure of surface energy, water adhesion tension (τ) defined as $\tau = \gamma \cos \theta$ ($\gamma =$ water surface tension, $\theta =$ contact angle) is often used. Relatively more water wettable surfaces with lower θ and higher τ are termed hydrophilic (high surface energy) surfaces, while less wettable surfaces with higher θ and lower τ are hydrophobic (low surface energy) surfaces. Although these are relative terms, the

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criterion of ca. $\theta = 65^\circ$ ($\tau = 30$ dyne/cm) that differentiates between the two regimes has been suggested [3,9–11].

It has been recognized that hydrophilic surfaces are generally favorable for short-term cell adhesion and proliferation for various cell types. Fibroblastic and endothelial cells adhered to a greater degree on substrata with higher surface energies [12,13]. Human trabecular osteoblastic cells also displayed increased attachment on stoichiometric hydroxyapatite that shows higher surface energy than on type A carbonate apatite with relatively lower surface energy [14]. Human epithelial cells displayed lower attachment on relatively more hydrophobic *N*-isopropylacrylamide copolymer films, and this trend corresponded to changes in fibronectin adsorption [15]. On the other hand, ROS 17/2.8 osteoblast-like cell attachment did not display a positive correlation with the non-dispersive component of surface energy [16]. In our previous study [3], human fetal osteoblastic (hFOB 1.19, hFOB) cell response to entire water wettability window was examined using a series of flat model surfaces with $\theta = 0^\circ - 115^\circ$, wherein hFOB cells displayed greater adhesion and proliferation on hydrophilic surfaces with $30 < \tau < 73$ dyne/cm. We also demonstrated that hFOB cells cultured on hydrophilic substrata displayed greater expression in selective integrins (αv, β3, and αvβ3 but not α2, α3, α4, α5, or β1) relative to cells on hydrophobic substrata [4]. Taken together, it can be concluded that surface energy and resultant wettability significantly affect short-term anchorage-dependent cell functions.

In contrast to these short-term effects, there is limited information regarding later stage surface energy effects on cell behavior. Some studies have recently reported long-term surface energy effects on bone cells. For example, primary human osteoblastic cells displayed increased collagen production after 6 week culturing on high surface energy hydroxyapatite relative to cells on low surface energy carbonate apatite [14]. Zhao et al. [17] showed that MG63 osteoblastic cells grown on hydroxylated, high surface energy Ti surfaces exhibited a more bone-like phenotype, e.g., increased alkaline phosphatase activity and osteocalcin, through higher production of PGE_2 and TGF-β1. We examined in this study whether surface energy affects osteoblastic cell growth and mineralization behavior using a medium-term culture (up to day 21). We utilized hFOB cells cultured on surfaces with two extreme surface energies, fully water wettable and very poorly water wettable. Cell morphology was observed by optical, electron, and confocal microscopy techniques, and matrix mineralization was examined by using mineral staining techniques and infrared spectroscopic techniques. As supplemental experiments, we screened whether other types of osteoblast-like cells, MC3T3-E1, MG63, and SAOS-2, displayed similar effects in cell morphology with respect to surface energy.

2. Materials and methods

2.1. Hydrophilic and hydrophobic surfaces

Fully water wettable surfaces were produced by plasma-discharge-treatment of monolithic quartz. Quartz slides (75.0×25.4 mm^2) were washed in distilled water, isopropyl alcohol, and chloroform and plasma-treated at 13.56 MHz and 100 mtorr in an inductively coupled plasma cleaner (Harrick). To produce hydrophobic surfaces, quartz slides were first plasma-treated and then reacted with octadecyltrichlorosilane (OTS) at 5% (w/v) solution in chloroform at an elevated, reflux temperature. OTS-treated substrata were rinsed with chloroform to remove non-reacted OTS, minimizing contact with air, and then air-dried.

Water contact angle of test surfaces was measured using an automated contact angle goniometer as previously described [3]. Additionally, Wilhelmy balance tensiometry was performed using a commercial computer-controlled instrument (Camtel CDCA 100, Royston). The balance was calibrated with standard weights thereby accounting for local variation in the force of gravity. No attempt was made to thermostat the balance and all measurements were made at ambient temperature. Distilled, deionized water (18.2 MΩ cm, approximately 10 ml) was used as the test solution and was contained in disposable polystyrene beakers previously determined not to measurably affect interfacial tension of water contained therein. Advancing and receding contact angles were calculated from the last of three immersion and emersion force measurements, respectively, using a force-balance equation corrected for buoyancy (by extrapolation to zero volume); $f = P\gamma \cos\theta$, where f is the force, P is the perimeter (wetted length) of the quartz substrate (54.8 mm, for a width of 25.4 mm and a thickness of 2 mm), γ is the water surface tension of 72.8 dyne/cm, and θ is either advancing or receding contact angle.

2.2. Cell culture

hFOB cells were maintained using Dulbecco's modified Eagle's medium–Ham's F-12 1:1 media (GIBCO) supplemented with 10% v/v fetal bovine serum (HyClone) and 1% v/v penicillin–streptomycin (GIBCO). For cell culture assays, cells were removed by applying a trypsin–ethylenediaminetetraacetic acid solution and cultured on test substrates at 1×10^4 cells/cm^2 using the same media further supplemented with 100 μg/ml ascorbic acid (Sigma), 10^{-8} M 1,25-dihydroxy vitamin D_3 (Biomol), and 10^{-8} M menadione (Sigma). A standard incubator (37 °C, 5% CO_2) was used. For sterilization, substrata were exposed to ultra violet light for 1 h before cell culture. Media were changed every 3–4 days.

2.3. Scanning electron microscopy (SEM) of cultured cells

After culturing 14 and 21 days, cells were fixed in a 2.5% w/v glutaraldehyde solution in phosphate buffered saline (PBS). Fixed cells were washed with PBS and dehydrated by consecutively adding ethanol/distilled water mixtures containing 50, 80, 90, 95, 98, and 100% volumes of ethanol. Samples were sputter-coated with gold and observed by FEI-Philips XL-20 SEM at an accelerating voltage of 20 kV. For hFOB cells forming clump-like structures on hydrophobic surfaces, at least 10 clump structures were observed by SEM.

2.4. Clump number and size measurement

Number and size of hFOB clumps formed on hydrophobic surfaces were quantified after culturing 14 and 21 days. Cells were fixed with a 4% w/v paraformaldehyde solution in PBS, dried, and observed by optical microscope. Clump number was visually counted. Clump size was quantified based on the number of calibrated pixels covered by the clumps using ImageJ image analysis software. Clump number per unit surface area ($n = 4$ quartz slides) and average clump size ($n =$ total 145 and 383 clumps for day 14 and 21, respectively) were assessed.

2.5. Confocal microscopy of immunofluorescent nuclei staining

On day 21, cells were fixed with a 4% w/v paraformaldehyde solution in PBS, rinsed with a 0.05% v/v tween-20 wash buffer, and permeabilized with a 0.1% v/v Triton X-100 solution. After blocking with a 1% w/v bovine serum albumin solution in PBS, cells were exposed to 4′,6-diamidino-2-phenylindole (DAPI, Chemicon, Fak100-90229) and observed using a Leica TCS SP2