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Surface-templated hydrogel patterns prompt matrix-dependent migration of breast cancer cells towards chemokine-secreting cells

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ABSTRACT

This paper describes a novel technique for fabricating spatially defined cell-laden collagen hydrogels, using patterned, non-adhesive polyacrylamide-coated polydimethylsiloxane (PDMS) surfaces as a template. Precisely patterned embedded co-cultures of breast cancer cells and chemokine-producing cells generated with this technique revealed matrix-dependent and chemokine isoform-dependent migration of cancer cells. CXCL12 chemokine-secreting cells induce significantly more chemotaxis of cancer cells when the 3-D extracellular matrix (ECM) includes components that bind the secreted CXCL12 chemokines. Experimental observations using cells that secrete CXCL12 isoforms with different matrix affinities together with computational simulations show that stronger ligand–matrix interactions sharpen chemoattractant gradients, leading to increased chemotaxis of the CXCL12 gradient-sensing CXCR4 receptor-expressing (CXCR4+) cells patterned in the hydrogel. These results extend our recent report on CXCL12 isoform-dependent chemotaxis studies from 2-D to 3-D environments and additionally reveal the important role of ECM composition. The developed technology is simple, versatile and robust; and as chemoattractant-matrix interactions are common, the methods described here should be broadly applicable for study of physiological migration of many different cell types in response to a variety of chemoattractants.

1. Introduction

Gradient formation and sensing is a complicated process involved in many physiological and pathological processes. Cells will change morphology and move toward a chemical gradient depending on the shape, dynamics and magnitude of the gradient [1]. The simplest model of gradient formation involves diffusion of soluble factors away from cells that secrete them. However, in vivo there are two types of microenvironmental interactions that define gradient formation and sensing: ligand–matrix and ligand–cell. Several studies have demonstrated the importance of complex gradient shape and dynamics in vivo, driven by ligand–matrix interactions [2,3] and by active cell-dependent ligand scavenging [4]. Hence, to properly model and experimentally manipulate these complex chemotactic processes, we need experimental systems that recreate these environmental influences on gradient formation. In this work, we develop a technique that enables robust and versatile definition of in vitro multicellular/microenvironment interactions, in physiologically relevant 3-D environments, and utilize this technique to study the relationship between ligand–matrix and ligand–cell interactions on migration of breast cancer cells.

A few in vitro assays exist to recreate how ligand–matrix and ligand–cell interactions collectively guide gradient formation. Transwells [5], hydrogels [6–10] and microchannels [11–16] are typically used to spatially pattern cells, define morphogenetic and chemotactic gradients, and monitor cell morphology and chemotaxis. Although these assays isolate individual aspects of gradient formation and sensing, they fail to replicate how multiple cell types and matrix interactions together define a gradient. We previously developed an experimental source-sink system to replicate the formation of defined soluble gradients between spatially

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patterned cells that secrete ligands (source) and cells that scavenge ligands (sink) [17,18]. These previous studies capture the involvement of multiple cell types in source-sink gradient formation and the role of ligand binding to the device- and cell-surfaces. However, the relatively small surface area with limited amount of binding sites available in this simple 2-D assay is not sufficient to address the potential influence of ligand–matrix interactions, as compared to the in vivo situation in which 3-D matrices provide a significantly greater concentration of binding sites.

In this work, we develop a novel patterning system to spatially pattern cells within an extracellular matrix (ECM), creating a model tissue-like environment for studies of directional cell migration. There are several techniques to create desired hydrogel patterns, such as laser lithography [19–25] and microchannel guides [26–28], that often require significant expertise, specialized instruments and complicated processing. Our approach enables the precise positioning of multiple cell types within a 3-D matrix, using relatively simple tools and expertise that should be accessible to most wet-labs. As a first application of this technology, we spatially pattern cells engineered to secrete the α- and β-isoforms of the CXCL12 chemokine and CXCR4 + cells that respond to CXCL12, while varying the composition of the surrounding matrix. Using this breast cancer model system, we demonstrate (i) the ability to systematically control ligand–matrix interactions via matrix composition; (ii) the ability to spatially pattern multiple interacting cell types within a 3-D matrix; and (iii) the effects of ligand–matrix interactions on gradient formation, and on subsequent cell migration.

2. Materials and methods

Unless otherwise stated, all chemicals and reagents for cell culture were purchased from Sigma–Aldrich, fluorescent dyes from Invitrogen, and all other equipment and materials from Fisher Scientific.

2.1. Cell culture

MDA-MB-231 (231, ATCC) cells were primarily used for these experiments, and were cultured in fully supplemented Dulbecco's modified Eagle's medium (DMEM, with 10% fetal bovine serum (FBS), 1% antibiotics–antimycotics). Some demonstration experiments were conducted with NIH 3T3 murine fibroblasts (cultured in fully supplemented DMEM) and human bone marrow endothelial cells (HBMECs, a gift from Irma de Jong, cultured in fully supplemented endothelial cell growth media (EGM2) with 5% FBS; Lonza). Standard trypsinization-based subculture protocols were used to passage cells prior to the experiment. We previously described culture, lentiviral transduction and migration of 231 cells expressing CXCR4 towards 231 cells secreting CXCL12 [17,18]. Briefly, we transduced 231 cells sequentially with a CXCR4-GFP fusion [29] and NLS-AcGFP to facilitate receptor-based migration and image-based tracking of nuclei [18], respectively. We expressed CXCL12-isoforms fused to *Gaussia* luciferase (GL) upstream of the fluorescent protein mCherry in a pLVX IRES vector, to facilitate proportional fluorescence sorting for CXCL12-expressing cells [18].

2.2. Preparation of PAA-coated PDMS surface

A 10:1 (w/w) degassed mixture of polydimethylsiloxane (PDMS) and a curing agent was diluted with toluene (PDMS:toluene = 1:3). 12 mm diameter glass slides were dipped into the mixed solution and baked at 120 °C for 30 min. A 9:1 (v/v) mixture of prepolymer solution (18.9 wt.% acrylamide, 0.33 wt.% pluronic F108 and 80.77 wt.% water) and photoinitiator solution (0.3 wt.% benzophenone and 99.7 wt.% 2-propanol) were added to the PDMS-coated slides and polymerized under UV (CL-1000, UVP: 8 W × 4 min) followed by an extensive wash in water [30,31]. The polyacrylamide (PAA)-coated PDMS slides were stored at ambient conditions and used for following hydrogel patterning within a few days (Fig. 1A).

2.3. Fabrication of oxidized patterns

The SU-8 master molds were fabricated by soft lithography. The detailed procedure for fabrication of master molds can be found elsewhere [32]. The PDMS replicas of patterns which served as oxidation stencils were placed in conformal contact with the PAA-coated PDMS slides and oxidized (100 W × 10 min) [33]. Oxidized PAA-coated PDMS slides were immediately used for hydrogel patterning (Fig. 1B).

2.4. Surface characterization

Water contact angles in air were measured on PDMS-coated slides and PAA-coated PDMS slides before and after oxidation by the sessile drop technique (4 μl of water) using a goniometer and analyzed with ImageJ. Data are expressed as a mean ± standard deviation (n = 3). Surface topography of PAA-coated PDMS slides oxidized through patterns was measured using a Bruker Veeco atomic force microscope in ScanAsyst mode, and analyzed with a Nanoscope (Veeco, Bruker).

2.5. Hydrogel patterning

2 ml of degassed PDMS was poured and cured into each well of a 6-well plate (Fig. 1C). Each PDMS well was punched along the shape of a 12 mm glass slide and each oxidized PAA-coated PDMS slide was set at the bottom of the well. Trypsinized cells were mixed with neutralized type I bovine collagen (BD Biosciences) to create a suspension of 10 million cells ml^{-1} in 2 mg ml^{-1} of collagen. For cell-free experiments, 1 μm diameter fluorescent beads (Sigma) were added to the neutralized collagen solution. 6 μl of the collagen gel solution was dispensed over each adhesive pattern and allowed to polymerize for 45 min in a humidified cell incubator (37 °C, 5% CO2). 250 μl of either 2.5 mg ml^{-1} neutralized collagen or 2.5 mg ml^{-1} neutralized collagen supplemented with a growth factor reduced Matrigel (BD Biosciences: 61% laminin, 30% collagen IV, 7% entacin and 2% other proteins including proteoglycans) mixture of collagen gel and matrigel solutions (75 vol.% collagen I + 25 vol.% Matrigel) was poured on each well and allowed to polymerize for 1 h in the humidified incubator. 2 ml of cell culture media were added to each well and cultured at 37 °C. For observation of non-specific cellular movement, Latrunculin (10 μM) was added to the media in control samples to prevent actin polymerization and hence migration.

2.6. Cell adhesion test

DMEM cell culture media containing MDA-MB-231 cells was cultured on tissue culture plastic, PAA-coated PDMS or oxidized PAA-coated PDMS slides in a 24-well plate and incubated for 24 h. Cell culture media was aspirated and samples were rinsed twice in PBS. 1 ml of 4% paraformaldehyde solution was added to fix cells. Cellular actin cytoskeletal structures and the cell nucleus were labeled with Phalloidin and Hoechst dyes, following protocols obtained from the manufacturers. Images were collected with an epifluorescent microscope (TE-300, Nikon) and analyzed with ImageJ to determine cell density and spread area, expressed as a mean ± standard deviation (n = 6).

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