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Bi-ligand surfaces with oriented and patterned protein for real-time tracking of cell migration

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ABSTRACT

A bioactive platform for the quantitative observation of cell migration is presented by (1) presenting migration factors in a well-defined manner on 2-D substrates, and (2) enabling continuous cell tracking. Well-defined substrate presentation is achieved by correctly orienting immobilized proteins (chemokines and cell adhesion molecules), such that the active site is accessible to cell surface receptors. A thiol-terminated self-assembled monolayer on a silica slide was used as a base substrate for subsequent chemistry. The thiol-terminated surface was converted to an immobilized metal ion surface using a maleimido-nitrilotriacetic acid (NTA) cross-linker that bound Histidine-tagged recombinant proteins on the surface with uniform distribution and specific orientation. This platform was used to study the influence of surface-immobilized chemokine SDF-1α and cell adhesion molecule ICAM-1 on murine splenic B lymphocyte migration. While soluble SDF-1α induced trans-migration in a Boyden Chamber type chemotaxis assay, immobilized SDF-1α alone did not elicit significant surface-migration on our test-platform surface. Surface-immobilized cell adhesion protein, ICAM-1, in conjunction with activation enabled migration of this cell type on our surface. Controlled exposure to UV light was used to produce stable linear gradients of His-tagged recombinant SDF-1α co-immobilized with ICAM-1 following our surface chemistry approach. XPS and antibody staining showed defined gradients of outwardly oriented SDF-1α active sites. This test platform can be especially valuable for investigators interested in studying the influence of surface-immobilized factors on cell behavior and may also be used as a cell migration enabling platform for testing the effects of various diffusible agents.

1. Introduction

Cell migration is fundamental to a wide variety of phenomena such as homing of lymphocytes to lymphoid organs [1], movement of leukocytes toward sites of infection [2], tissue morphogenesis [3], movement of metastatic cells toward sources of growth factors [4], orchestration of neuronal wiring during brain development [5], angiogenesis [6], and wound healing [7]. The incessant "random" migration of B lymphocytes within lymphoid organs, evolved to maximize the probability that rare antigen-specific B cells encounter their cognate antigen, [8,9] is tightly regulated via micro-anatomical localization, differentiation states, receptor engagement, and coordinated interactions of adhesion molecules and chemokines, making them migratory only under specialized conditions [10,11]. It is not surprising then that since the late 1800s investigators have consistently noted the difficulty of B lymphocytes to migrate on 2-D surfaces in vitro that lack some of these key elements [12–15].

Lymphocyte migration studies have typically used [16] the Boyden Chamber type transmigration chemotaxis assays [17] that have several limitations [18]. First, they lack the ability to dissect the roles of autocrine and paracrine signaling. Second, they do not allow discernment of cell migration parameters such as cell displacement, track length, translocation speed, directional persistence time, chemotactic/haptotactic index, and turning behavior because this assay-type monitors a population of cells after exposure to a chemoattractant in a steep gradient across a very thin porous mesh, a process which is not directly viewable and thus allows data acquisition only at the end points of experiments. Third, this type of

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Table 1
Different approaches to immobilize proteins on surfaces.

Immobilization	Mechanism	Orientation	Stability	Ref.
Adsorption	Hydrophobic, hydrogen-bonding, van der Waals, and/or electrostatic interactions of proteins with surface	Random	Poor	[27]
Assembly	Hydrophobic interactions create hybrid lipid bilayers that present protein on surface	Random	Poor	[28]
Covalent binding	Lysine-residues reaction with N-hydroxysuccinimide (NHS) ester groups on surface	Random	Excellent	[29]
	Lysine-residues reaction with aldehyde groups on surface	Random	Excellent	[30]
	Cysteine-residues reaction with maleimide groups on surface	Random	Excellent	[31]
	Cysteine-residues reaction with disulfide-derivatized surface	Random	Excellent	[32]
Affinity	Biotin-tag interaction with streptavidin on surface	Oriented	Excellent	[33]
	His-tag interaction with metal-ion chelated surface	Oriented	Good	[34]
	Leucine zipper-tag interaction with complementary zipper on surface	Oriented	Good	[35]
	Glutathione S-Transferase (GST)-tag interaction with glutathione on surface	Oriented	Good	[36]
	Fc-tag interaction with Protein A or Protein G on surface	Oriented	Good	[37]

assay is prone to the influence of interfering artifacts and is less conservative at distinguishing between chemotaxis and chemokinesis because the pore size and thickness of the trans-migration mesh/membrane are of the same order of magnitude as the characteristic dimension of the migrating cell body. Finally, they do not allow the study of the effects of surface-immobilized factors such as chemokines and cell adhesion molecules (either solo or concurrently with other immobilized and diffusible factors) on cell migration.

Zigmond [19] and Dunn [20] chambers and other approaches including the ibidi® cell migration slide/chambers have been developed and used [21–24] to directly visualize the cell migration process via time-lapse imaging on a 2-D substrate, enabling researchers to avoid some of the limitations of the Boyden Chamber type assays. Much like the Boyden Chamber though, the Zigmond and Dunn Chamber methods utilize a quasi-static diffusive gradient (that is sensitive to fluid flow fluctuations), and are not optimized for the presentation of surface-immobilized factors that influence cell migration. Perhaps cell migration researchers have yet to embrace techniques developed for immobilizing and orienting protein, as has been done in other fields, particularly biosensors, proteomics, protein adsorption, and cell adhesion [25,26]. Table 1 summarizes some of the techniques developed to immobilize proteins on surface.

Because proteins have complex structures and activities, an immobilization approach that preserves a protein's native state and orients it for optimal target interactions over an extended period of time would be ideal. Past attempts on protein immobilization have mainly used nonspecific adsorption [27,28], or covalent bond formation between available functional groups (e.g., amines, thiols) on protein molecules and complementary coupling groups (e.g., aldehyde, maleimides) on the surface [29–32]. A major concern with both these approaches is the random orientation of proteins on the surfaces [25,26]. This precludes the active sites of a substantial population of immobilized protein molecules from being accessible to targets such as cell surface receptors [38,39]. In addition, the possibility of protein denaturation exists upon strong interaction between randomly immobilized protein and the surface [26,39]. Although, the covalently attached protein is more permanently bound as compared to the physisorbed protein, the latter also risks desorption [25,40]. Therefore, surface-techniques to control the orientation and stability of immobilized protein molecules on solid surfaces would be very useful [25,26,41,42]. To solve this problem, affinity based interaction such as streptavidin–biotin [33], leucine zipper tags [35], Glutathione–Glutathione S-Transferase (GST) tag [36], Nickel-His-tag [34], Protein A-Fc tag [37] have been applied in the past.

Developing a much needed robust in vitro test-platform for studying the migration of "fickle" cell types such as B lymphocytes applying some of these techniques from surface science would be useful. In this study a cell migration testing platform was developed that (1) utilized surfaces of defined chemistry to stably present an adhesive protein [8,43] in conjunction with a chemokine [10,44] (proteins associated with B lymphocyte migration) in an orientation that promotes interactions with the cognate cell receptor, (2) enabled direct and continuous visualization of cell migratory behavior, (3) showed amenability of this surface immobilization approach to patterning via UV to form surface gradients of proteins of interest in a tunable fashion. As proof-of-principle this platform cell culture substrate was used to probe the effect of surface-immobilized chemokine SDF-1α and cell adhesion molecule ICAM-1 on the migration of B lymphocytes.

2. Materials and methods

2.1. Fabrication and characterization of Histidine-tagged SDF-1α

2.1.1. Expression plasmid vector and bacterial transformation

A pET32 plasmid encoding C-terminal 6X His-tagged SDF-1α was kindly provided by Dr. Ghalib Alkhatib from Texas Tech University Health Science Center. 50 μl of BL21(DE3)pLysS cells (Strategene, Santa Clara, CA) was transformed with 50–100 ng of plasmid and grown on antibiotic selective agar plates (100 μg/ml carbenicillin, BioPioneer Inc., San Diego, CA).

2.1.2. Recombinant protein expression, purification, and refolding

A bacteria culture originating from a single colony on the agar plate was grown in LB broth with 100 μg/ml carbenicillin at 37 °C until an OD600 of 0.6 was achieved. Culture was then induced with 1 mM isopropyl β-D-thiogalactoside for 5 h at 30 °C and protein expression was confirmed via SDS-PAGE.

The bacterial pellet was harvested via centrifugation and resuspended in lysis buffer to break open the bacterial cell wall and membrane. The suspension was rotated (30 min, RT), sonicated (50% duty and 60 pulses/cycle for 3 cycles), rotated again (30 min, RT), and centrifuged (15,000 × *g* for 20 min at 4 °C). The pellet was resuspended in 6 M guanidine in base phosphate buffer to solubilize the inclusion bodies containing the recombinant His-tagged SDF-1α protein, rotated, sonicated, and rotated again. The lysate was centrifuged and protein supernatant was mixed overnight at 4 °C with a column of primed Ni–NTA agarose (Qiagen, Germantown, MD).

The next day, the protein–agarose mixture was washed with decreasing guanidine concentration (6 M, 4 M, 2 M, 0 M) pH 7.8 in base phosphate buffer and 20 mM imidazole, respectively. The protein was then eluted with 0.5 M imidazole in base phosphate buffer (pH 7.2) and diluted with Round 1 refolding buffer (55 mM Tris–Base, 10 mM NaCl, 0.4 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$,