Biomaterials 32 (2011) 2634–2641

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Controlled architectural and chemotactic studies of 3D cell migration

Prakriti Tayalia [a], Eric Mazur [a,b], David J. Mooney [a,c,*]

[a] School of Engineering and Applied Sciences, Harvard University, 9 Oxford Street, Cambridge, MA 02138, United States
[b] Department of Physics, Harvard University, 9 Oxford Street, Cambridge, MA 02138, United States
[c] Wyss Institute, Harvard University, 9 Oxford Street, Cambridge, MA 02138, United States

ARTICLE INFO

Article history:
Received 29 September 2010
Accepted 13 December 2010
Available online 14 January 2011

Keywords:
Live imaging
Dendritic cells
Two-photon polymerization
Microfabrication
Scaffolds
CCL19 gradient

ABSTRACT

Chemotaxis plays a critical role in tissue development and wound repair, and is widely studied using *ex vivo* model systems in applications such as immunotherapy. However, typical chemotactic models employ 2D systems that are less physiologically relevant or use end-point assays, that reveal little about the stepwise dynamics of the migration process. To overcome these limitations, we developed a new model system using microfabrication techniques, sustained drug delivery approaches, and theoretical modeling of chemotactic agent diffusion. This model system allows us to study the effects of 3D architecture and chemotactic agent gradient on immune cell migration in real time. We find that dendritic cell migration is characterized by a strong interplay between matrix architecture and chemotactic gradients, and migration is also influenced dramatically by the cell activation state. Our results indicate that Lipopolysaccharide-activated dendritic cells studied in a traditional transwell system actually exhibit anomalous migration behavior. Such a 3D *ex vivo* system lends itself for analyzing cell migratory behavior in response to single or multiple competitive cues and could prove useful in vaccine development.

1. Introduction

Concentration gradients of bioactive signaling molecules regulate processes ranging from embryonic development[1] to wound repair [2], as cells express directional locomotion directed by these gradients in a process termed chemotaxis. Commonly used chemotactic assays include Boyden chamber [3], under-agarose assay [4], Zigmond chamber,[5,6], Dunn chamber [7], and micropipette assay [8]. These assays, although inexpensive and easy to use, have several limitations; they restrict observation of cell migration to two dimensions, do not allow one to monitor the dynamics of migration and do not sustain the signaling gradients for more than a few hours [9]. For example, the Boyden chamber, which is an endpoint assay, does not allow observation of chemotaxis visually, and cannot directly distinguish chemotaxis (oriented locomotion) from chemokinesis (stimulated nondirected or random locomotion). Various confounding variables, including variations in the pore size and thickness of membranes used in this assay further obscure the migratory response of cells to chemokines [10,11]. Variations to existing systems allow for the development of gradients with better stability[12,13]. Microfluidics-based methods for generating stable concentration gradients have recently been developed [14,15], but they typically expose cells to shear flow, which can bias directional motility[16] and inadvertently dilute autocrine/paracrine factors secreted by the cells. Current microfluidic chemotaxis chambers also limit studies of cell migration to two-dimensional (2D) substrates, but many relevant biological processes including immune cell migration and cancer cell invasion, involve migration in a three-dimensional (3D) environment.

A particularly important example of 3D cell migration is the chemotaxis of dendritic cells (DCs). DCs act as sentinels for the immune system, with immature dendritic cells patrolling the body to seek out foreign agents such as bacteria, viruses, or toxins. The dendritic cells bind, ingest, and present fragments of antigens on cell surface receptors; in addition, encounters with antigens also trigger dendritic cell maturation (or activation), which enables these cells to home to the lymph nodes in response to chemotactic agents (chemokines) secreted from the node [17]. The upregulation of chemokine receptors upon the activation of DCs allows chemotactic migration of these cells [18]. Specifically, mature dendritic cells upregulate CCR7, a chemotactic factor receptor that binds the lymph node-derived chemokines CCL19 and CCL21 [18,19]. Expression of CCR7 enables the directed migration of these cells to the spleen, or through the lymphatic system to a lymph node, where they present antigens to other immune cells, and generate an immune response to the antigen [20,21]. Maturation also involves upregulation of other cell-surface receptors including CD80 (B7.1), CD86 (B7.2), and CD40, that act as

* Corresponding author. School of Engineering and Applied Sciences, Harvard University, 9 Oxford Street, Cambridge, Massachusetts 02138, United States.
E-mail address: mooney@deas.harvard.edu (D.J. Mooney).

0142-9612/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2010.12.019

co-receptors and enhance T-cell activation [17]. In spite of the importance of dendritic cell migration to proper immune system function and successful DC-based anti-tumor therapy, quantitative understanding of 3D dendritic cell migration is currently quite limited.

In this paper, we describe an approach to simultaneously study the influence of chemotactic signals and 3D architectural features of an adhesion substrate on directed 3D dendritic cell migration. We used two-photon polymerization to fabricate 3D interconnected scaffolds with precise architectural control at the micrometer scale. A sustained release system is integrated into a microfabricated chamber to generate chemotactic gradients in the scaffolds containing cells. We developed a theoretical model to obtain the concentration, release and gradient profiles of chemokines and to predict a range of scaffold architectural parameters that either promote or restrain the motion of cells in response to a chemokine gradient. Finally, a live imaging and analysis system permits us to study chemotactic cell migration within the scaffolds, and to directly test how architectural features regulated chemokine-driven chemotaxis.

2. Materials and methods

2.1. Primary cells (DCs) isolation and culture

A protocol developed by Lutz *et al* [22]. was adopted for generation and purification of primary bone-marrow-derived dendritic cells (BMDCs). Briefly, bone marrow cells were flushed from the femurs of green fluorescent protein-expressing (GFP) C57BL/6 mice and cultured in 100-mm bacteriological petri dishes (Falcon number 1029/Becton Dickinson). Cell culture medium RPMI-1640 (R10) (Sigma) was supplemented with 1% Penicillin-Streptomycin (Invitrogen), 2 mM L-Glutamine (Invitrogen), 50 μM 2-mercaptoethanol (Sigma) and 10% heat-inactivated fetal bovine serum (FBS, Invitrogen). At day 0, bone marrow leukocytes were seeded at 2×10^6 cells per 100-mm dish in 10 ml R10 medium containing 20 ng/ml granulocyte-macrophage colony-stimulating factor (GMCSF) (Peprotech). At day 3 another 10 ml R10 medium containing 20 ng/mL GMCSF was added to the plates. At days 6 and 8, half of the culture supernatant was collected and centrifuged, the cell pellet was resuspended in 10 ml fresh R10 containing 20 ng/mL GMCSF, and placed back into the original plate. We used the non-adherent cell population in the culture supernatant between days 8 and 12 for all our experiments.

2.2. Transwell migration

Transwell migration studies were performed by plating cells in the top well, and adding chemokine/adjuvant to the bottom well underneath the porous membrane. 600 μL of media with 300 ng/mL of CCL19 (Peprotech) was added to the bottom wells. 3×10^5 cells suspended in 100 μL of conditioned media were added to the top transwell. Three cell conditions were tested: (1) unstimulated cells, (2) cells stimulated with condensed 5 μg/mL CpG (Invivogen) (with 1 μg/mL prostaglandinE2 (PGE2), from Cayman Chemical) and (3) cells stimulated with 1 μg/mL LPS (Sigma). The number of cells that migrated from the top well to the bottom well through the porous membrane was counted at the end of 12 h to quantify migration. Cells that had migrated to the bottom well were collected by treatment with 0.25% trypsin-0.03% ethylenediaminetetraacetic acid (EDTA, Invitrogen) and counted with a Z2 coulter counter (Beckman Coulter, Inc.).

2.3. FACS staining and sorting

CpG was condensed with polyethyleneimine (PEI, Sigma)[23] to decrease its size and enhance its uptake by cells. As PGE2 has been reported to enhance dendritic cell activation and migration *in vitro*[24,25] it was used in conjunction with CpG activation. For activation, a combination of 5 μg/mL of condensed CpG and 1 μg/mL of PGE2, or 1 μg/mL of LPS was used.

For fluorescence activated cell sorting (FACS) characterization, cells were stimulated with CpG/PGE2 or LPS for 24 h, and then collected (3×10^6 cells for each condition) to stain and quantify for surface presence of the costimulatory molecule CD86. For staining, the cells were washed and resuspended in a 'wash medium' consisting of phosphate buffered saline solution with 10% FBS (Invitrogen) and 1% sodium azide (Sigma). Using 0.06 μg of antibody per million cells in a 100-μL volume of wash medium, cells were incubated with a fluorophore tagged anti-CD86 molecule (Ebioscience) for 30 min over ice on a shaker. The cells were washed thrice in wash medium and FACS analysis was used to quantify the number of fluorescently labeled cells for different stimulatory conditions. The percentage activation was measured against the isotype control, to account for non-specific staining.

To separate the 100% activated cell population from their unactivated counterparts, the cells were preactivated with CpG for 24 h. They were then stained with CD86 antibody as described above and sorted using FACS machine to collect both positive and negative populations.

2.4. Fabrication of L-shaped device

An L-shaped device for studying chemotaxis was made using a combination of two-photon polymerization and polydimethylsiloxane (PDMS) molding. First, two-photon polymerization was used to fabricate a scaffold using triacrylate polymer on a coverglass as described in detail in our previous work [26]. Briefly, the triacrylate monomer was placed on a coverglass and polymerized using a focused femtosecond laser beam. The unpolymerized monomer was washed off using a solvent to yield a three-dimensional interconnected scaffold. The scaffolds were 600 μm long, 600 μm wide and about 100 μm high. Then PDMS walls 1 mm in height by 1 mm in width, as shown in Fig. 1, were replicated from an L-shaped mold made from plastic. The center of middle chamber of the L-shaped device was aligned with the center of the scaffold under a microscope and the walls were stuck to the cover glass with the scaffolds. Each chamber in the L-shaped device was 3 × 3 mm.

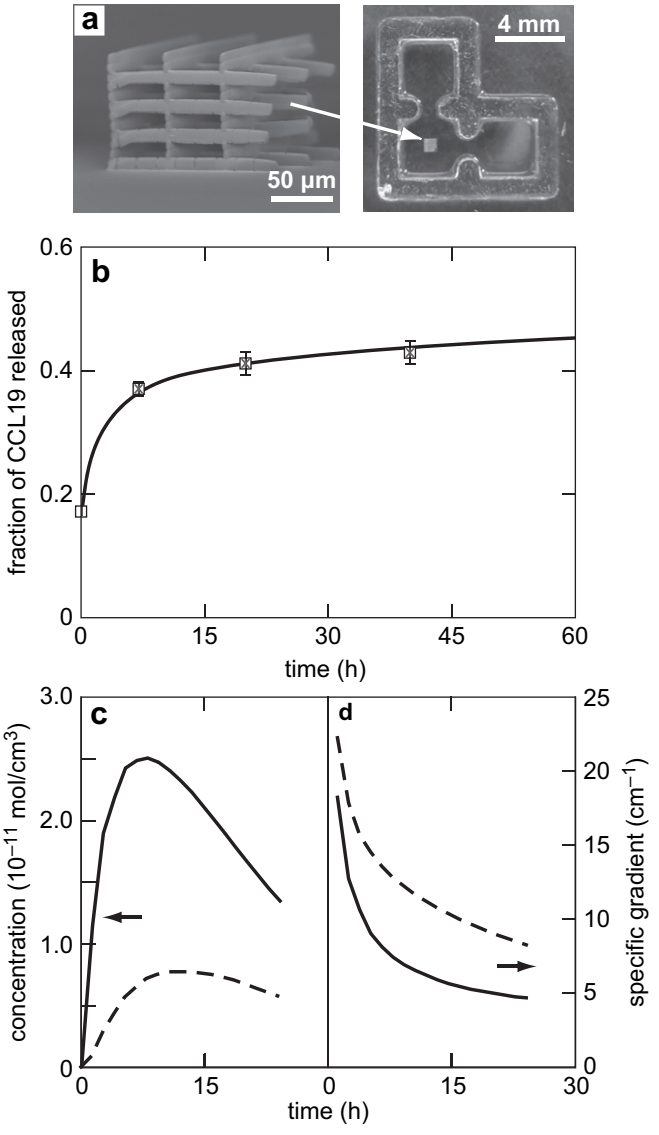

Fig. 1. Development of migration system. (a) Left: Scanning electron micrograph (SEM) of two-photon polymerized scaffold showing its interconnected and porous three-dimensional structure; right: brightfield image of a PDMS-molded, L-shaped device with a two-photon polymerized scaffold in the center; (b) amount of chemokine released from collagen gels, measured as a fraction of total chemokine incorporated; time dependence of the (c) concentration and (d) specific gradient of the chemokine in the center of the scaffold (solid line) and at the left edge of the device (dashed line), as predicted by finite element modeling.