Biomaterials 31 (2010) 2686–2694

Contents lists available at ScienceDirect

Biomaterials

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

Convection-driven generation of long-range material gradients

Yanan Du [a,b,1], Matthew J. Hancock [a,b,1], Jiankang He [a,b,c,1], Jose L. Villa-Uribe [a,b], Ben Wang [a,b], Donald M. Cropek [d], Ali Khademhosseini [a,b,*]

[a] Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA 02139, USA
[b] Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[c] State Key Laboratory of Manufacturing Systems Engineering, Xi'an Jiaotong University, Xi'an, Shaanxi 710049, China
[d] U.S. Army Corps of Engineers, Construction Engineering Research Laboratory, Champaign, IL 61822, USA

ARTICLE INFO

Article history:
Received 21 November 2009
Accepted 3 December 2009
Available online 24 December 2009

Keywords:
Anisotropic materials
Composite materials
Microfluidics
Gradients

ABSTRACT

Natural materials exhibit anisotropy with variations in soluble factors, cell distribution, and matrix properties. The ability to recreate the heterogeneity of the natural materials is a major challenge for investigating cell–material interactions and for developing biomimetic materials. Here we present a generic fluidic approach using convection and alternating flow to rapidly generate multi-centimeter gradients of biomolecules, polymers, beads and cells and cross-gradients of two species in a microchannel. Accompanying theoretical estimates and simulations of gradient growth provide design criteria over a range of material properties. A poly(ethylene-glycol) hydrogel gradient, a porous collagen gradient and a composite material with a hyaluronic acid/gelatin cross-gradient were generated with continuous variations in material properties and in their ability to regulate cellular response. This simple yet generic fluidic platform should prove useful for creating anisotropic biomimetic materials and high-throughput platforms for investigating cell–microenvironment interactions.

1. Introduction

Anisotropic materials are highly important for many natural and engineered systems. Examples of anisotropic materials in nature include marbles, tree trunks and squid beaks. Examples of engineered anisotropic materials include the birefringent crystals of prisms, the metal wood head of golf clubs and the aluminum alloys used in aircraft and rockets. Spatial anisotropy in materials is especially prominent in cellular microenvironments *in vivo* where heterogeneous distributions of cells and molecules exist within spatially varying extracellular matrices (ECM). Molecular concentration gradients play an important role in biological phenomena such as chemotaxis, [1,2] morphogenesis and wound healing [3–5]. Meanwhile, the graded variations of ECM and cell concentration at the tissue interface (e.g. bone–cartilage interface, dentino–enamel junctions) are nature's solution for connecting mechanically mismatched tissues [6,7]. Creating chemical and material gradients to mimic the heterogeneity of cellular environments is important for investigating cell–matrix interactions [8] and for developing biomimetic materials for tissue engineering [9].

Various methods exist to generate molecular and material gradients (Supplementary Table 1). Diffusion-based approaches for gradient generation are limited to diffusible molecules and require long times to create millimetric gradients, since the timescale for pure diffusion scales as length squared. Dispersion-based approaches, which combine primary stretching by flow shear and secondary spreading by diffusion, have been used to generate centimeter long molecular gradients in seconds to minutes [10–12]. However, so far no generic platform employing dispersion to generate material gradients of single or multiple components over long distances has been developed. In this study, we present a generic microfluidic dispersion-based platform for rapidly (seconds to minutes) generating long-range material gradients of molecules, polymers, particles and cells. By using a syringe pump to drive fast alternating flows which continually lengthen the gradient, we had, to our knowledge, for the first time created centimeter scale concentration gradients of cells and microbeads by flow convection. Our work is also the first to generate cross-gradients in particles and hydrogels by using alternating flows to superpose gradients of two species. In particular, we have generated composite materials containing a 'cross-gradient' of two

* Corresponding author. Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA 02139, USA. Fax: +1 617 768 8477.
E-mail address: alik@rics.bwh.harvard.edu (A. Khademhosseini).
[1] These authors contributed equally to this work.

0142-9612/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2009.12.012

hydrogels or two types of microbeads. Our simple yet versatile gradient platform (Fig. S1) should prove useful for a wide range of applications that involve anisotropic material gradients.

Fluidic shear-driven stretching, also known as convective or hydrodynamic stretching, is the primary mode of gradient generation in the present work. In short, a particle in the center of the channel moves faster than one at the wall and the two spread apart at a rate proportional to the maximum channel velocity. A gradient so forms in the laterally averaged concentration profile (Fig. 1A). Ironically, diffusion acts to suppress hydrodynamic stretching by reducing the mean variation in particle speeds: [13,14] slowly moving particles near the wall diffuse toward the center and accelerate; while fast moving particles near the center diffuse toward the wall and decelerate (Fig. 1B). For dilute suspensions of micron sized and larger particles moving in viscous flows, diffusion is negligible [15,16]. However, negatively buoyant particles settle under gravity to the channel bottom (Fig. 1C), whereupon all particles experience the same low velocity and spreading ceases.

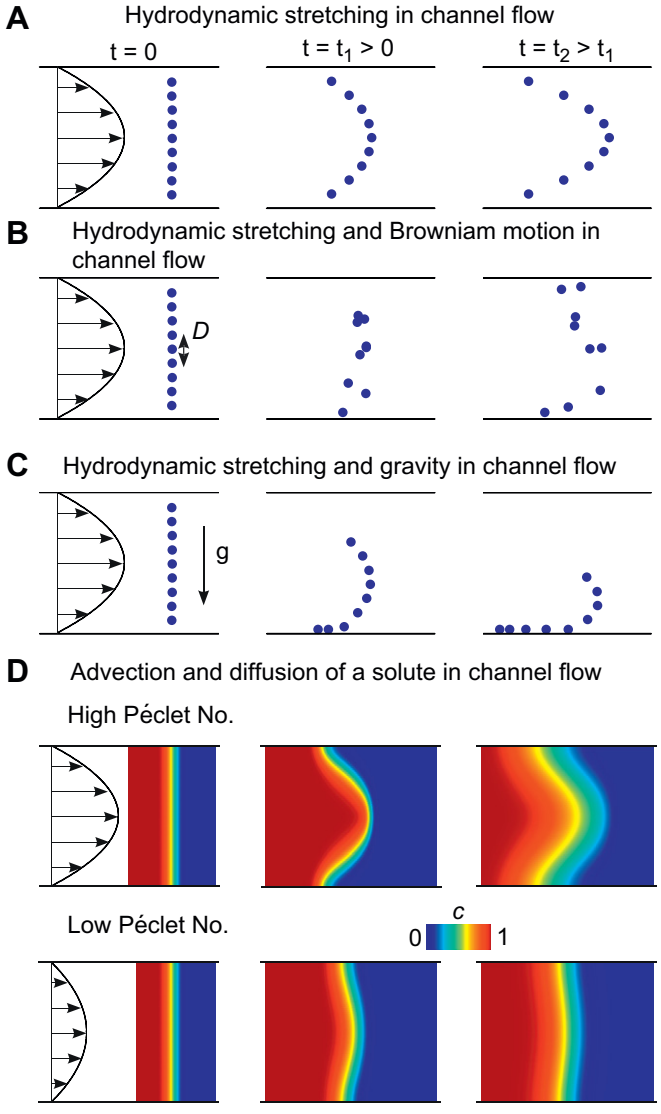

Fig. 1. Physical picture of gradient generation in channel flow. A. Particle spreading due to convection (hydrodynamic stretching). B. Vertical diffusion characterized by a diffusion coefficient D suppresses longitudinal convection-driven spreading. C. Gravitational settling suppresses longitudinal convection-driven spreading. D. Advection and diffusion of dissolved solute in high/low Péclet flows.

Thus, high flow rates improve stretching at all scales: for molecules, high flow rates dominate diffusion which acts to suppress hydrodynamic stretching; for microparticles such as microbeads and cells, high flow rates are imperative to spread the particles before they settle. The latter may explain why centimeter scale gradients of micron sized particles have not been previously generated by convection in microchannels. In the following sections, we demonstrate that high-speed (mm/s) flow shear-driven stretching can generate gradients of a wide range of species (molecules, cells, microbeads) along a simple microchannel.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol-diacrylate) (MW 4000) was purchased from Monomer–Polymer & Dajac Labs. The photo-initiator (PI), 2-hydroxy-1-[4-(hydroxyethoxy)-phenyl]-2-methyl-ι-propanone (Irgacure D2959), was purchased from Ciba Geigy (Dover, NJ). Polyethylene microtubing (I.D. 0.38 mm, O.D. 1.09 mm) was purchased from Intramedic Clay Adams (Becton Dickinson & Co, MD). Green Fluorescent FITC-microbead and non-fluorescent microbead solutions were purchased from Polysciences (Warrington, PA). Human Umbilical Vein Endothelial cells (HUVECs) and endothelial cell basal medium (EBM-2, Clonetics) supplemented with 0.5 mL vascular endothelial growth factor (VEGF), 0.2 mL hydrocortisone, 0.5 mL epidermal growth factor (rhEGF), 0.5 mL ascorbic acid, 2.0 mL r-human fibroblast growth factor-B (rhFGF-B), 0.5 mL heparin, 0.5 mL recomb long R insulin-like growth factor (R3-IGF-1) and 0.5 mL gentamicin sulfate amphotericin-B (GA-1000) were obtained from Lonza (Basel, Switzerland). All other reagents were purchased from Sigma–Aldrich (St. Louis, MO) unless otherwise indicated.

2.2. Fabrication of microchannel

The microchannel was fabricated by a standard soft lithography method described previously [10] and consisted of a top polydimethylsiloxane (PDMS) fluidic channel that was plasma bonded onto a bottom glass slide. The rectangular channel dimensions were 100 μm (height) × 2 mm (width) × 50 mm (length).

2.3. Generation of biomolecule gradient

The microchannel was pre-filled with 1× Dulbecco's Phosphate Buffered Saline (DPBS) solution. 1 wt% fluorescein isothiocyanate-dextran (FITC-dextran, MW 10 kDa) solution was sequentially pumped (forward flow) and withdrawn (backward flow) into the channel at flow rates between 0.007 and 0.044 ml min^{-1} with a syringe pump (World Precision Instruments Aladdin 1000, WPI, FL). Forward and backward flows were separated by 30 s of downtime. Two flow sequences were used, alternately pumping and withdrawing fluid in the channel: 4.7, 2.0, and 1.3 μL (three flow segments); 5.2, 3.5, 2.9, 2.4, 2.1, 1.8, and 1.6 μL (seven flow segments). Flow rates were calibrated with a flow meter from Gilmont Instruments, IL. The imaging protocol is outlined in Supp. II.4. The diffusion coefficients for several MWs of FITC-dextran dissolved in PBS at 25 °C have been measured [17] and give similar results to one study of FITC-dextran in water [18]. Averaging interpolated results from four studies [17–20] that measured the diffusion coefficient D of 10 kDa FITC-dextran in water and PBS at 25 °C yields $D = 1.3 \times 10^{-6}$ cm^2 s^{-1}. The particular values interpolated from each study ranged from 0.9 to 2.0 × 10^{-6} cm^2 s^{-1} due to differences in the degree of branching and polydispersity of the dextrans used in the studies [17].

2.4. Generation of bead/cell gradients

Microbead stock solutions containing microbeads with diameters 5.0 and 10 μm (with a solid fraction of 0.1% w/w) were diluted 10 times in DPBS. 6 μL of the microbead solution was pumped at a rate of 0.044 ml/min into the channel, followed by 30 s of downtime. Subsequent pumping did not alter the gradient. The protocol for generating cell gradients was similar to that for the microbead gradients. HUVECs were cultured in endothelial cell basal medium at 37 °C in a humidified incubator. HUVEC medium was used in place of DPBS as the background solution and medium containing HUVECs (5×10^6/ml) after trypsinization was used in place of the microbead solution.

2.5. Generation of PEG-DA hydrogel gradient

The channel was pre-filled with 5 wt% PEG-DA solution. A concentration gradient of hydrogel precursor solution (with high concentration of 40 wt% PEG-DA in DPBS and 1% PI) was generated at a flow rate of 0.025 ml/min using the flow sequence outlined above for FITC-dextran. The hydrogel precursor concentration gradient was cross-linked via photo-polymerization (UV exposure: 10 mW/cm^2 for 20 s). For characterization, the resultant hydrogel was air-dried, cut in half with