Available online at www.sciencedirect.com
ScienceDirect
J. Biochem. Biophys. Methods 70 (2008) 1192–1195
JOURNAL OF biochemical and biophysical methods
www.elsevier.com/locate/jbbm

Short note

Fabrication of protein gradients for cell culture using a miniature squeegee

Santiago Costantino [a,*,1], Christopher G. McQuinn [b,1], Timothy E. Kennedy [c], Paul W. Wiseman [a,b]

[a] McGill Program in NeuroEngineering, Department of Physics, McGill University, Montréal, Québec, Canada
[b] McGill Program in NeuroEngineering, Department of Chemistry, McGill University, Montréal, Québec, Canada
[c] McGill Program in NeuroEngineering, Department of Neurology and Neurosurgery, McGill University, Montréal, Québec, Canada

Received 8 March 2007; received in revised form 25 May 2007; accepted 29 May 2007

Abstract

We present a straightforward method to create spatial gradients of substrate bound protein for live cell studies using only mechanical parts. Protein concentration gradients on a micron scale can be fabricated in several minutes for a relatively low cost using a method that is generally applicable to any protein and substrate combination. We describe the details of the device construction, and provide examples of mammalian cells grown on substrates patterned with protein concentration gradients using this technique.

Keywords: Protein gradient; Protein patterning; Axon guidance; Substrate modification

1. Introduction

The aim of reproducing in vitro the spatial distributions of proteins found in vivo during key events in the development of organisms requires simple and reliable ways to fabricate protein concentration gradients. Axonal guidance and cell migration [1–5] are two essential processes in which spatial gradients of chemoattractants and chemorepellents play a fundamental role. Here we report a method to fabricate substrate bound protein gradients in a straightforward way using only mechanical parts to make the patterns with a resolution of several micrometers.

Technologies allowing precise patterning of proteins at a cellular scale have become the focus of a growing field of research [6] and there has been a constant increase in their applications in biology, both as a tool for basic research [7,8] and also for commercial purposes [9]. Several techniques have already proven useful to create such protein patterns, but none of the reported methods has successfully emerged as a standard. Furthermore, the physical characteristics of different proteins of interest are highly variable, and it is likely that one patterning technique will not be ideal for all proteins. Tradeoffs between the length scales of the patterns to be generated, reproducibility, cost of the necessary equipment and consumables, and ease of use of the available techniques has left the door open to the development of new instruments and methods.

Initial methods that aimed to create gradients of membrane proteins in order to determine their influence on axon guidance in vitro were limited in their capacity to reproduce the steepness and small scale required to mimic gradients of interest found in vivo [5,10]. Photonic techniques offer a promising alternative [11], but the use of UV-lasers and specialized chemical cross-linkers that are not commercially available entail that these methods are not easily accessible to typical biomedical research laboratories. The precise deposition of nanodrops of protein solution [12] and microfluidic devices [13] were also applied to address this challenge, but require specialized equipment and have not been widely used in subsequent studies. Recently, gradients of protein spot densities were fabricated by soft lithography of ephrin-6, and these geometric patterns were shown to influence axon extension by retinal neurons [14]. This latter method allowed the generation of well defined graded patterns at a macroscopic scale across distances of tens of micrometers, however a weakness of the technique is that the

* Corresponding author. 3600 University Building, Montreal, QC, Canada, H3A 2T8.
E-mail address: santiago.costantino@mcgill.ca (S. Costantino).
[1] These authors contributed equally to this work.

0165-022X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbbm.2007.05.013

local concentration of protein within each spot remains constant throughout the whole structure while the density of spots is varied.

2. Materials and methods

The device we present consists of a barrier squeegee made of hydrophobic material that is used to spatially confine a protein solution within a substrate binding area set by the location of the squeegee, and which controls the incubation time by mechanical translation of the barrier. A motorized translation stage is used to precisely move this silicone barrier thus exposing the different areas of the substrate to the protein solution in user defined increments in space and time. By varying the incubation time, or by depleting the protein solution, it is possible to control the coverage of the substrate to achieve a graded pattern, within certain limits that are set by the adsorption kinetics that are protein specific. A photograph of the device is shown in Fig. 1D.

After sequential translation of the mechanical barrier, the first area where the drop of protein solution is placed will adsorb the largest number of proteins as it has the longest exposure (reaction) time. As well, the last exposed area will have the lowest surface concentration of the protein. The differences in adsorbed protein concentration in different regions of the substrate result from two contributing factors. As new areas of the substrate surface are exposed to the solution, the concentration of the protein in solution will decrease thus yielding lower numbers of molecules available to bind to the surface. Additionally, incubation time will also contribute to the binding kinetics. The incubation period of the first step of the gradient is equal to the sum of all incubation times ($t_1+t_2+t_3\ldots+t_n$) for all steps in generating the gradient pattern. The second step is incubated for the entire time that it takes to expose all of the remaining areas ($t_2+t_3\ldots+t_n$) in the stepped pattern and so forth.

The horizontal movement of the squeegee that confines the liquid was performed using a motorized translation stage (Thorlabs, Newton, NJ) and custom routines written in LabVIEW (National Instruments, Austin, TX). The DC motor we used has a built-in optical encoder with a large number of counts per revolution that provides submicron positioning accuracy. The chosen translation velocity (300 μm/s) and the step dwell times are inputs of the program.

The pressure that the squeegee exerts on the substrate has to be adjusted depending on the flexibility of the silicone barrier in order to obtain reproducible patterns. The force applied by the barrier is controlled with a second translation stage and a screw. In order to measure this force, the experiments are performed on a simple laboratory balance with the downward force adjusted to approximately 5 g. The squeegees were made of PDMS (poly (dimethylsiloxane), Dow Corning, Midland, MI) due to its versatility and low cost. A mixing ratio of 10:1 of silicone prepolymer to curing agent was used and was placed in an oven

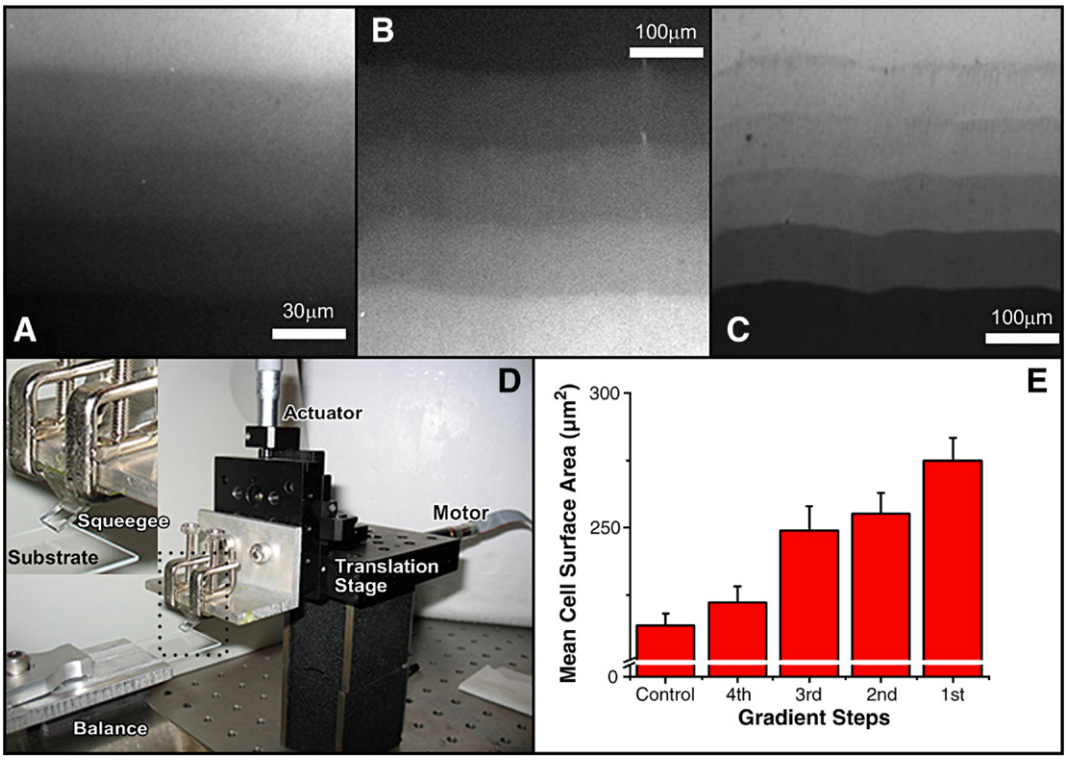

Fig. 1. Three examples of protein gradients at different scales are shown. A and B) are mixtures of fibronectin and Alexa546-human fibrinogen on aldehyde activated slides and C) Alexa488-goat-antimouse antibodies. D) A photograph of the patterning device, including a close up of the PDMS squeegee near the slide surface. E) CHO-K1 cells expressing EGF receptors/GFP were cultured on graded patterns of fibronectin. The cell surface spreading was quantified and shown as cell area function of protein concentration.