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Tuning cell–surface affinity to direct cell specific responses to patterned proteins

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ABSTRACT

Interactions with local extracellular cues direct cell migration. A versatile method to study cell response to a protein consists of patterning the protein cue on a substrate and quantifying the distribution of cells between patterned and non-patterned areas. Here, we define the concepts of (i) cell–surface affinity to describe cell choices, and of (ii) reference surface (RS) to clarify that the choice is made relative to a reference. Furthermore, we report a method to systematically tune the RS and show that it can dominate the experimental cell response to a protein cue. The cell response to a cue can be switched from strong preference to strong aversion by only changing the RS. Using microcontact printing, we patterned the extracellular matrix proteins fibronectin or netrin-1 adjacent to a series of RSs with different ratios of poly-D-lysine (PDL) and polyethylene glycol (PEG), which are of high affinity and of low-affinity for cells, respectively. C2C12 myoblasts or primary neurons seeded on substrates with a high affinity RS (high % PDL) did not respond to a printed protein of interest, and conversely on RSs of low affinity (high % PEG) the cells preferred the printed protein even in the absence of a specific interaction. However, when testing cell response to a standardized series of RSs varying from high to low affinity, a specific response curve was obtained that was unique to each cell type–protein pair. Importantly, for intermediate RSs with moderate affinity, the cell response to the cue was dependent on the activation of biologically relevant protein-specific biochemical signal transduction pathways. Our results establish that choices made by cells in response to a surface-bound cue must take into account, and be interpreted in the context of, the RS. The use of a series of RSs with varying cell–surface affinity reveals specific response curves of cells to a cue that can be compared quantitatively and that may help gain new insights into cellular responses to extracellular proteins.

1. Introduction

Cell migration *in vivo* is a complex process in which multiple extracellular cues may be integrated to evoke a cellular response [1]. Interactions with cues surrounding the cell activate signaling pathways that regulate adhesion and migration [2]. Adhesion is essential for many forms of cell migration and is regulated through transmembrane adhesion proteins that bind to ligands presented on cell surfaces or components of the extracellular matrix (ECM), such as fibronectin and laminins [3]. Contact-mediated adhesion and signaling trigger the assembly of intracellular macromolecular complexes around the site of adhesion that transmit both mechanical force and regulatory signals to the cytoskeleton [4]. Using similar mechanisms, gradients of secreted chemotropic guidance cues like netrin-1 [5] promote directional cell migration, in part through a mechanism dependent on cell–substrate adhesion [6].

In order to study the mechanisms involved in contact-mediated cell responses, *in vitro* assays have been developed that employ patterned substrate-bound proteins to challenge cells with precise spatial distributions of molecular cues [7,8]. While these assays fall

Abbreviations: PLL-g-PEG, poly-L-lysine-grafted-polyethylene glycol; PDL, poly-D-lysine; PDMS, polydimethylsiloxane.

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short of duplicating the complexity of the *in vivo* environment, they can succeed in isolating the influence of one or a limited number of extracellular cues [9]. Cells preferentially attach and migrate onto surfaces due to a combination of different adhesion and signal transduction mechanisms that reorganize the cytoskeleton [10]. While the strength of the adhesive interaction plays an important role, cells sometimes prefer a less adhesive surface. Laminin-1, for example, was shown to be less adhesive than fibronectin, but cells subjected to a choice assay between both surfaces preferably moved onto the laminin pattern [11]. Thus, in consideration that higher adhesion does not always equate preference, we use the descriptive term "cell–surface affinity". Although this term had been used previously in the context of cell choice assays for neurons between two surfaces, neither the meaning nor the underlying concept were well-defined, and its functional significance has not been investigated relative to the contiguous surface [12–15].

In addition to affinity, we introduce the concept of a reference surface (RS) and report a method to tune the cell–surface affinity of the RS. The surface contiguous to a patterned protein has a widely accepted but rarely discussed role in the responses cells make to patterned protein substrates. Fig. 1 shows that the cell response can be switched from strong affinity to strong aversion simply by tuning the RS.

Whereas it is expected that the cell response depends on the RS, many manuscripts fail to report the composition of the RS or how it was produced [16]. Although its importance has not been broadly recognized, the RS should be considered as it will help to qualify the findings, compare studies conducted in different laboratories, and account for discrepancies. Despite cells typically having a low affinity for untreated glass or polystyrene, and therefore preferentially adhering to almost any protein substrate [17], such RSs have often been used in studies of cell migration. For instance, a polystyrene RS was used to examine the response of neurons to a patterned grid of the laminin peptide IKVAV [18]. To better control the RS, cell culture surfaces are often coated with poly-D-lysine (PDL) [19] and polyethylene glycol (PEG) [20–22] that are known for their very high and very low affinity to cells, respectively. For example, a RS of polylysine was used to study neurite preference between laminin and fibronectin in a juxtaposed stripe assay and migration on substrate-bound netrin-1 gradients [23,24]. Conversely, a RS made of PEG was used to investigate neurite outgrowth on patterns of RGD peptide and fibronectin [25]. In these two studies, since one of the surfaces had a high affinity and the other a low affinity, one might expect that the RS could influence the outcome and the conclusions made. Another study, which examined the capacity of melanoma cells to form sites of adhesion on geometric arrangements of fibronectin and vitronectin surface-bound dots, used a RS formed of hydrophilic alkanethiols that may have acted as a cell repellent, akin to glass [26]. Another case had EphrinA5 gradients formed with a RS of perpendicular laminin tracks (a high affinity surface) to demonstrate repulsion of RGD neurons [27]. Increasing concentrations of EphrinA5 would be expected to mask the laminin, an effect that may have contributed to responses that were solely attributed to EphrinA5. The absence of understanding and control of the RS may have confounded the design and conclusions of certain studies in more subtle ways as well. For example, Evans et al. used microchannels to flow different concentrations of fibronectin and laminin to form stripes on a substrate, followed by backfilling with a fixed concentration of poly-L-lysine (PLL), in effect serving as the RS [23]. The higher affinity of the neurons for PLL may have masked their response to fibronectin and laminin, and the use of a RS with a high affinity may have prevented the use of lower concentrations of fibronectin and laminin, which could have led to a stronger differential response and a more convincing result.

Here, in addition to clarifying and formally defining the concepts of cell–surface affinity and RS, we also present a novel standardized protocol to tune the RS by using mixtures of PDL (high affinity) and PLL-g-PEG (low affinity, named PEG hereafter for brevity) with varying ratios of %PEG:%PDL ranging from 0:100 (highest affinity) to 100:0 (lowest affinity). We tested the set of RSs in a stripe assay [28] with lines of either fibronectin or netrin-1 patterned using microcontact printing [29] surrounded by a specific %PEG:%PDL ratio in each experiment. We characterized the adhesion, spreading, migration and focal adhesions of cells on various RSs, and observed that for intermediate cell–surface affinity of the RS, both migratory and polarized cells (*i.e.* neurons) respond in a physiologically appropriate manner to the patterned surface-bound proteins. For extreme affinity values, *e.g.* highest and lowest affinity, cells universally adhere to or avoid the patterned protein cues, respectively, indicating that the RS dominates the response and has the capacity to mask specific cellular responses. It follows that optimal RS composition can be selected to maximize the capacity of a particular cell type to respond to a printed protein of interest via ligand–receptor interactions. A graphical representation of the cell response to a protein as function of the RSs yielded an affinity "binding curve" that was specific for each cell and each surface. This affinity curve not only constitutes a quantitative and reproducible way of characterizing the cell response to a cue but also creates new defined parameters for measuring cellular responses including magnitude, range and slope of the response.

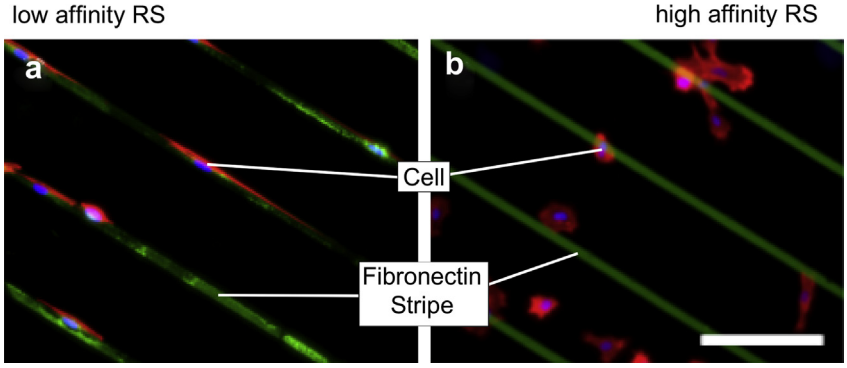

Fig. 1. Cell response to fibronectin stripes on a RS with high affinity (typically adhesive) or low affinity (typically non-adhesive). Illustrative images of Rat2 fibroblasts responding to stripes of substrate-bound fibronectin protein (green) showing the differences in cell response obtained by altering the RS to widely different levels of cell–surface affinity. Cells were stained with phalloidin to visualize F-actin (red) and Hoechst to label nuclei (blue). (a) On non-adhesive RSs, cells preferentially stick to the cue lines whereas (b) on adhesive surfaces they stick to the RS. Scale bar is 100 μm.