Cell discrimination among simultaneous presentation of substrate-bound and soluble protein gradients

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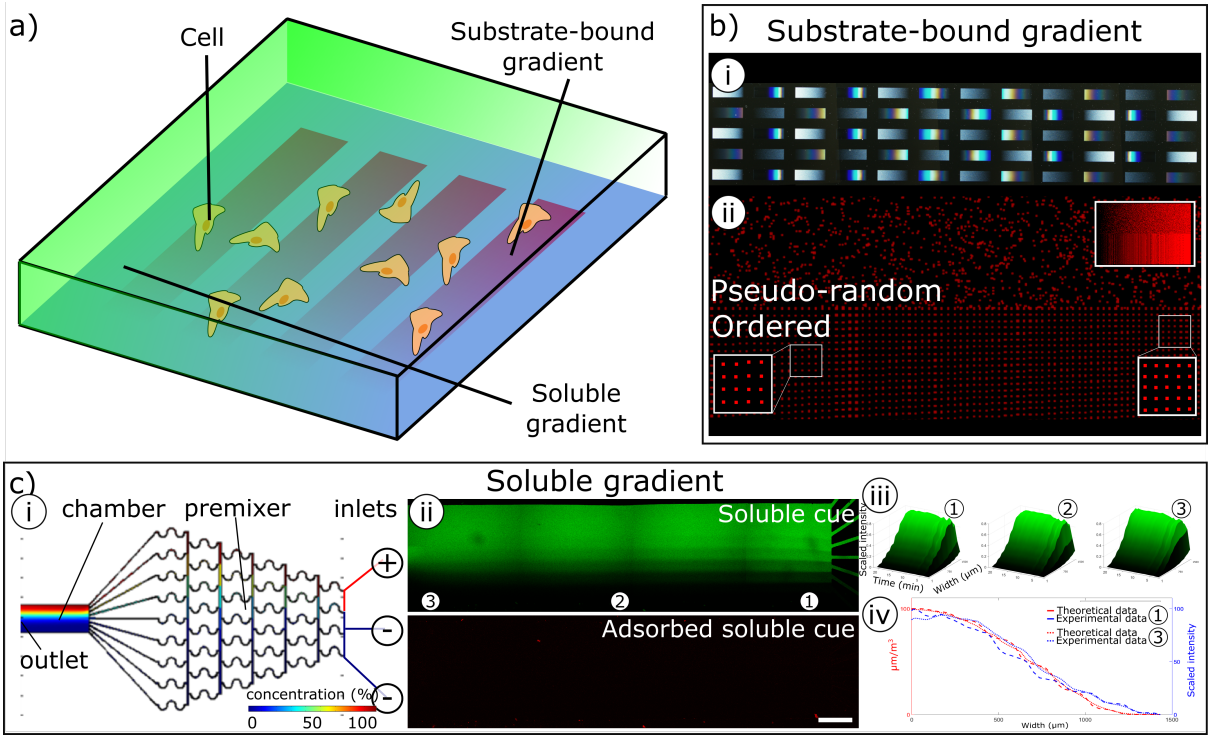
To overcome the extracellular matrix's (ECM) complexity, cells rely on a plethora of cues to reach specific targets. Cues are concurrently presented to cells, and the cells must discriminate amongst these cues through a mechanism yet to be understood. To study cell navigation, in vitro methods have been developed to create gradients of single cues, such as substrate-bound or soluble proteins. Although insightful, these approaches lack the ability to present cells with multiple cues of various types. Here, we present a platform which for the first time enables the study of cell discrimination between soluble and substrate-bound protein gradients.



(a) Schematic of the device fabricated composed of substrate-bound and soluble gradients to tease apart the cell response to different cues simultaneously presented to cells. (b) Substrate-bound protein gradients achieved through nanocontact printing. (i) Image and (ii) design of the substrate-bound gradient array composed of 60 distinct gradients made up of 200 nm dots arranged either orderly or pseudo-randomly. Insets show full gradient as well as magnified sections of the ordered gradient. (c) (i) The microfluidic gradient generator is made up of 3 inlets, to mix three solutions in the premixer portion of the device and release the mixtures into the cell culture chamber. The concentration distribution was simulated in Comsol. (ii) Gradient characterization was confirmed experimentally with dextran and lack of binding of the soluble cue to the surface confirmed through immunostaining. (iii) Experimental characterization of the gradient shows that stabilization of the gradient occurs within 2 min of the cue addition. (iv) Gradient slopes calculated computationally and experimentally in regions 1 and 3 in the chamber show an overlap with an accuracy of R^2= 0.983 and 0.988, respectively. Result discrepancies could be result from inaccuracies of the diffusion rate input for the simulation. Scale bar is 500 μm.