Combinatorial Assay

Two-dimensional microfluidic methods have been used to to create complex overlapping gradients using pneumatic actuation[1,2] and a wide range of gradient profiles (*e.g.* linear, exponential, polynomial and step-wise) been demonstrated using flow based methods[3,4]. These methods are advantageous because of their ability to rapidly tune gradient characteristics by modulating flow rates or the frequency of valve actuation. However, the main disadvantages (in the realm of biologically relevant assays) is that they are 1) confined to 2D substrates, and 2) they require external equipment that reduces their ability to reach a widespread audience.

Here, we demonstrate how complex overlapping gradients can be created within a 3D gel and scaled to create combinatorial migration assays. A 100μm tall channel with photolithographically defined dosing windows is shown in Figure 1. Each window can operate as either a dosing window or a patterning widow where cells can be added. The resistance provided by the gel is critical in adding factors to the system without disturbing the environment. Complex gradients can be created by dosing a window with a desired factor. The window area and relative spacing determines the degree of overlap between factor. After the desired degree of overlap is achieved, cells can be added to the cell addition window and the response to the overlapping gradient can be observed in real time. Even though this approach is diffusion based, factors can diffuse quickly over short distances. For example a small molecule (D = 5x10^{-6}cm^2 sec^{-1}) can diffuse a distance of 10 microns in $\sim$0.1 second (assuming the factor does not react with the matrix) allowing for rapid changes to the microenvironment over cellular scale lengths.

In this way, the behavior of complex overlapping gradients can be studied in a combinatorial assay that can be scaled up as desired. Figure 1(a) shows how a simple system can be used to study the migratory behavior of cells exposed to 5 different complex gradients within the same experimental construct; this type of assay can be used to determine how cells respond to the physiologically relevant situation where multiple cues are present within a 3D environment gel and may lead to into a hierarchy of signaling factors. Figure 1(b) schematically demonstrates how experimental conditions and controls can be built into the the same system for combinatorial studies and easily scaled up. Cell migration assays created in this way are more structurally similar to the *in vivo* environment than traditional 2D assays and may lead to an increased understanding of the conditions that lead to both ideal and inappropriate immune cell recruitment.

References

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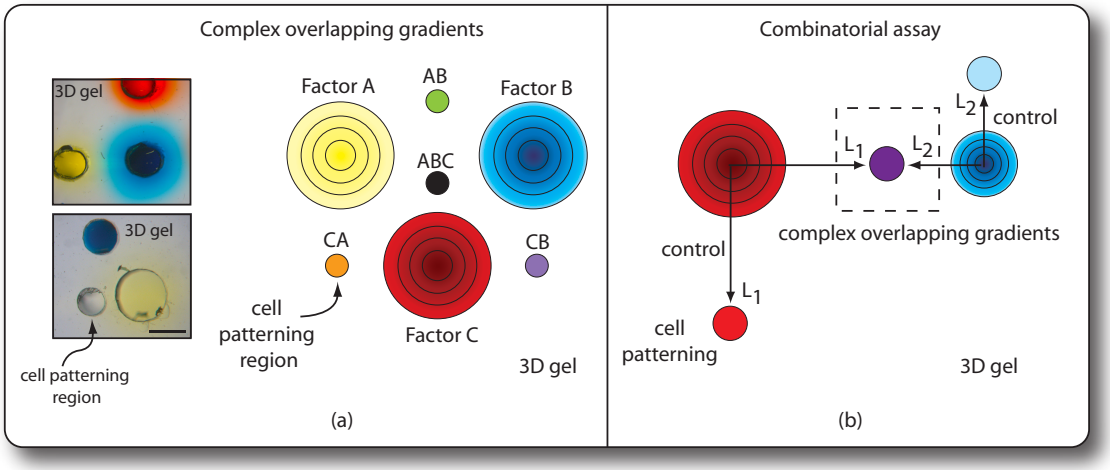

Figure 1: Schematic shows the ability to create complex overlapping gradients within a 3D environment. a) Different factors can be added to the dosing windows of a gel filled channel. The window size, separation distance, and addition time can be changed to create complex overlapping and opposing gradients. Migratory cell populations can then be added and the cellular response to multiple factors can be investigated. Scale bar = 1 mm. b) Demonstrates combinatorial assays that can be scaled up for high-throughput applications.