Stackable micropatterned hydrogels for analysis of thick tissues in vitro

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In vitro models of thick tissues are often difficult to maintain due to a limited supply of oxygen and nutrients to cells by diffusive mass transport. This limitation has sparked several methods of integrating pre-formed blood vessels that allow for convective mass transport throughout the tissue. Often these methods lack an ability to dictate the composition of the cells within the tissue and to isolate cells from specific regions of the tissue for analysis. The article by Son et al. [1] demonstrates how multi-layered stacks of cell-laden hydrogels can maintain viable cells by integrating micropatterned channels to allow flow to occur. The authors demonstrate that the multi-layered stacks of HepG2 cells have higher production rates of albumin and sustain longer secretion periods of urea than single layer cultures. This platform provides a versatile method of creating thick tissue-like constructs for the analysis of cells.

Tissues are complex 3D structures that often rely on the presence of concentration gradients, which are generated by the cells themselves [2, 3]. For example, the liver contains functional units, referred to as an acinus, which contains regions defined by the mass transport of oxygen from the afferent blood vessels [4]. These gradients are often absent from conventional 2D culture systems due to the immediate dilution of the secreted factors into the bulk culture medium, which is often many orders of magnitude larger than in the in vivo environment [5]. Spheroid cultures and microfluidic systems are two methods of culture that facilitate the presence of these gradients; however, isolating subpopulations of cells that reside in a specific region of the gradient for further analysis remains challenging.

A unique method for creating thick models of tissues is done by stacking thin layers of cells on top of each other. The advantage of this method is that the final composition of the thick tissue is simply determined by the order of the individual layers that are assembled. A successful implementation of this method is known as cell sheets [6], which uses a temperature sensitive protein, poly(N-isopropylacrylamide), to remove monolayers of cells from a culture dish for subsequent stacking either in vitro or directly in vivo. Although this method has been proven to be very effective for tissue engineering applications, two features of this technology limit its use for effective analysis of thick tissues in vitro, (i) the individual layers are a single cell thick, requiring many layers to be stacked to achieve truly thick (>1 mm) assemblies; (ii) the individual layers can’t be separated after culture, preventing isolation of cells within specific regions of the tissue.

The Whitesides lab developed a system, which addresses these two limitations, using sheets of paper as a scaffold to manually manipulate cell-laden hydrogels [7–10]. These paper-based individual layers enabled thick assemblies to be cultured in custom holders that intentionally restricted the diffusion of oxygen and nutrients to only the top of the stack of cells. These boundary conditions, coupled with the metabolism of the cells, resulted in the formation of monotonically decreasing gradients of oxygen that were used to recapitulate the pathologic environments of tumors and ischemic myocardium. Furthermore, the individual layers could be destacked for further analysis simply by peeling apart the layers of paper. To create thick healthy tissues, however, a means for convective mass transport is needed so that all layers can receive adequate amounts of oxygen and nutrients.

This article describes the formation of thick healthy tissues by integrating flow channels that can maintain the viability of the cells throughout the tissue. The flow channels are created by micropatterning holes into the individual layers, so that once aligned, the holes form channels that allow culture medium to flow through the tissue. In this article, the authors created an in vitro model of the liver, using HepG2 cells (see Figures in [1]). The results demonstrate that tissues of stacked layers produce higher levels of albumin and sustain secretion of urea for longer periods, compared to single layer cultures. Furthermore, microarray data suggests that cells in these stacked configurations down
regulate tumor-related genes. The authors also demonstrate that cocultures with NIH3T3 fibroblasts can be performed, although no data suggesting this improves liver function were presented. The demonstration provides evidence that the system is versatile and amenable for culturing cells from various organs, and potentially to guide stem cell differentiation. Further work that can be done on this system is to evaluate differences in gene expression based on zonal positioning within the acinus. The zonal boundaries should be controllable by modulating the flow rates through the tissue. Overall, this platform demonstrates a promising method for developing in vitro models of thick tissues for potential drug-screening and tissue engineering applications.

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References


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