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The role of endothelial cells in the retinal stem and progenitor cell niche within a 3D engineered hydrogel matrix

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ABSTRACT

Cell–cell interactions are critical to understanding functional tissues. A number of stem cell populations have been shown to receive key regulatory information from endothelial cells (ECs); however, the role of ECs in the retinal stem and progenitor cell (RSPC) niche has been largely unexplored. To gain greater insight into the role of ECs on RSPC fate, a three-dimensional (3D) co-culture model, incorporating cell–cell interactions, was designed by covalently-modifying agarose hydrogels with growth factors and cell-adhesive peptides in defined volumes. Therein ECs adopted tubular-like morphologies similar to those observed *in vivo*, but not observed in two-dimensional (2D) cultures. Unexpectedly, ECs inhibited proliferation and differentiation of RSPCs, revealing, for the first time, the possible role of ECs on RSPC fate. This 3D hydrogel scaffold provides a simple, reproducible and versatile method with which to answer biological questions related to the cellular microenvironment.

1. Introduction

In recent years, biomaterials have been designed as three-dimensional (3D) scaffolds in order to elucidate stem cell biology [1,2]. Ideally, bioactive materials will mimic the cellular microenvironment or stem cell niche, thereby providing insight into the spatial and temporal cues that govern stem cell fate [2–4]. One of the key considerations in stem cell niche research is interactions among different cell types [5]. Stem cell behavior is influenced by the presence of other cell types, either through direct cellular contact or secreted proteins, and results in differentiation and/or expansion and self-renewal [5,6]. Notwithstanding the importance of culturing multiple cell types in a 3D microenvironment, the relatively few co-culture studies that have been pursued to uncover mechanisms of cellular interactions, have been developed using two-dimensional (2D) cell culture [7–9]. Considering the functional and morphological differences of cells cultured in 2D vs. tissues [10], it is important to develop a 3D co-culture model that allows cells to adopt physiologically relevant phenotypes in order to gain greater insight into cellular interaction and function (Table S1).

Endothelial cells (ECs) have been shown to play fundamental roles in neurogenic niches beyond their traditional vascular roles as suppliers of oxygen and nutrients [11]. For example, neural stem and progenitor cells (NSPCs) in the subventricular zone (SVZ) of the brain are found in close proximity to capillary tips [12] where ECs have been shown to secrete factors, supporting neurogenesis of NSPCs [13,14]. The co-localization of ECs and stem/progenitor cells is not unique to the brain [5]; regulatory signals generated by ECs have also been shown to influence stem cell fate in various tissues, including adipose tissue [9,15] and the hematopoietic system [16,17].

Recent studies have demonstrated that the ciliary epithelium of the eye includes retinal stem and progenitor cells (RSPCs), showing the two cardinal properties of self-renewal and multipotentiality *in vitro* [18,19]. Similar to the NSPC niche, the RSPC niche consists of a highly dense 3D network of capillaries lined by ECs [20,21], suggesting a role for ECs in the RSPC niche. Interestingly, the role of ECs and RSPCs has largely gone unexplored. The RSPCs remain in the quiescent state *in vivo* and show no capacity for endogenous stimulation or regenerative capacity [18]. Elucidating whether ECs play a functional role as inhibitors of RSPC proliferation and differentiation is important to both better understand the cellular microenvironment and provide insight into treatment strategies of retinal degenerative disease. Interestingly, ECs in the microvasculature of the mouse brain [11,12,22] and ciliary body are both

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