Abstract

Stromal cells within lymphoid tissues are organized into three-dimensional structures that provide a scaffold that is thought to control the migration and development of haemopoietic cells. Importantly, the maintenance of this three-dimensional organization appears to be critical for normal stromal cell function, with two-dimensional monolayer cultures often being shown to be capable of supporting only individual fragments of lymphoid tissue function. In the thymus, complex networks of cortical and medullary epithelial cells act as a framework that controls the recruitment, proliferation, differentiation and survival of lymphoid progenitors as they undergo the multi-stage process of intrathymic T-cell development. Understanding the functional role of individual stromal compartments in the thymus is essential in determining how the thymus imposes self/non-self discrimination. Here we describe a technique in which we exploit the plasticity of fetal tissues to re-associate into intact three-dimensional structures \textit{in vitro}, following their enzymatic disaggregation. The dissociation of fetal thymus lobes into heterogeneous cellular mixtures, followed by their separation into individual cellular components, is then combined with the \textit{in vitro} re-association of these desired cell types into three-dimensional reaggregate structures at defined ratios, thereby providing an opportunity to investigate particular aspects of T-cell development under defined cellular conditions. (This article is based on work first reported Methods in Molecular Biology 2007, Vol. 380 pages 185-196).

Video Link

The video component of this article can be found at https://www.jove.com/video/905/

Protocol

For more information on preparing regaggregate thymus cultures please visit Springer Protocols.

Disclosures

The authors have nothing to disclose.