Science Education Collection

Invasion Assay Using 3D Matrices

URL: https://www.jove.com/science-education/5645

Abstract

The extracellular matrix (ECM) is a network of molecules that provide a structural framework for cells and tissues and helps facilitate intercellular communication. Three-dimensional cell culture techniques have been developed to more accurately model this extracellular environment for *in vitro* study. While many cell processes during migration through 3D matrices are similar to those required for movement across rigid 2D surfaces, including adherence, migration through ECM also requires cells to modulate and invade this polymeric-mesh of ECM.

In this video, we will present the structure and function of ECM and the basic mechanisms of how cells migrate through it. Then, we will examine the protocol of an assay for tube formation by endothelial cells, whose steps can be generalized to other experiments based on 3D matrices. We will finish by exploring several other biological questions that can be addressed using ECM invasion assays.

Transcript

Scientists have developed 3D models to more accurately study cell invasion and migration processes. While most traditional cell culture systems are 2D, cells in our tissues exist within a 3D network of molecules known as the extracellular matrix or ECM. While many of the mechanistic processes required for cell motility in 2D and 3D are similar, factors such as the reduced stiffness of ECM compared to plastic surfaces, the addition of a third dimension for migration, and the physical hindrance of moving through the mesh of long polymers in the ECM, all present different challenges to the cell compared to two-dimensional migration.

This video will briefly introduce the basic function and structure of the ECM, as well as the mechanisms by which cells modulate and migrate through it. Next, we'll discuss a general protocol used to study endothelial cell invasion. Finally, we will highlight several applications of 3D matrices to studying different biological questions.

Let's begin by examining the composition of the ECM, and how cells interact with it.

The ECM performs many functions, such as providing support for cells, facilitating intercellular communication, and separating tissues. ECM composition varies among different tissues and has different biological properties, but it can be classified into two broad types. The basement membrane serves to anchor and separate tissues, while interstitial matrix surrounds and supports the cells within a tissue. The interstitial matrix is mostly composed of the fibrous protein collagen, but also includes elastin and fibronectin.

Several biological processes need to occur for cells to migrate through the ECM. The first is cell-matrix adhesion, which involves transmembrane proteins called integrins. These link the ECM to the cell's internal scaffold, known as the cytoskeleton.

Another process is the structural rearrangement of the cell's cytoskeleton. This leads to the formation of specialized structures called invadopodia, which are protrusions of the cell into its surrounding matrix. The final step is ECM modulation. This typically involves degradative molecules known as matrix metalloproteases or MMPs, which accumulate in the invadopodia and degrade the surrounding ECM, facilitating cell invasion. 3D matrix invasion assays allow scientists to visualize and study this complex process.

Now that you're familiar with ECM and its interaction with cells, let's walk through a protocol for studying ECM invasion by endothelial cells to form tubules. By culturing endothelial cells in a 3D environment, one can simulate the biological process of blood vessel growth, also known as angiogenesis, which is important during both normal development, as well as cancer.

First, endothelial cells are cultured, and a single cell suspension is prepared by treating the cells with proteases such as trypsin, and passing them through a mesh filter to break up cell clumps. The 3D matrix, commonly composed of collagen, fibrin, laminin, or more complex combinations of these components—which can either be prepared in-lab or ordered from commercial vendors—is then thawed on ice. Since most ECM preparations polymerize at higher temperatures, it is helpful to keep other equipment and reagents cold as well. The cell suspension is mixed with the thawed matrix solution to embed cells, and this mixture is placed into a cell culture incubator where the higher temperature will cause the matrix to polymerize.

Once the cell-containing matrix is set, culture media containing angiogenic factors is added to the matrix dish. Using time-lapse microscopy software, individual cells can then be tracked to observe their migration through the matrix. The resulting images are analyzed, and cell positions are used to calculate movement direction and distance in microns. These values can then be plotted to determine locomotory activity—the average migration rate of the cells. Finally, tube network formation is observed and analyzed using visualization software to identify features such as nodes, tubes, and loops.

Now, let's explore a few applications of 3D matrices in specific experiments.

Cell migration is mediated by active modulation of the cellular cytoskeleton. In this experiment, collagen matrices were prepared and mixed with a stain containing red fluorescent protein to allow for visualization. Individual cell spheroids, which are free-floating cell clusters, were isolated and embedded in the collagen matrix. Following incubation, the embedded cells were stained for specific cytoskeletal components, and imaged by fluorescence microscopy. Researchers observed cytoskeletal components and their alterations as cells migrated through the ECM.

Scientists can also study how the properties of the ECM affect migration. Using a concentric gel system, where cells are embedded in an inner gel matrix surrounded by outer matrices of varying concentrations, scientists can track cells using time-lapse microscopy to study their migration from the inner gel to the initially cell-free outer gel. Researchers observed that the greater stiffness of higher concentration gels resulted in increases in both cell displacement and overall distance of cell migration.

Finally, matrix invasion assays can be performed within a living animal to study angiogenesis in an organ-specific context. Here, fibrin gels—commonly used in tissue engineering due to their biodegradable nature—were generated, followed by implantation into mouse lungs where the gels were held in place by a “glue” made of the protein fibrinogen. Cell migration and new blood vessel formation were allowed to occur for the following 7 to 30 days, after which the lungs and fibrin gels were harvested, fixed, and sectioned. Imaging of these sections revealed blood vessel and alveoli formation in the implanted gels, giving researchers insight into this crucial aspect of lung development in its *in vivo* setting.

You’ve just watched JoVE’s video on extracellular matrix invasion assays. This video discussed the composition of the ECM and how cells migrate through it, presented a simple protocol to study endothelial cell migration through a 3D matrix, and highlighted several cellular processes currently being studied in the context of cell-ECM interactions. Because endogenous cell migration occurs in 3D space, these biological conditions are best simulated by 3D culture techniques. Improvements in matrix composition will continue to allow scientists to more accurately replicate and study cellular migration in the lab. As always, thanks for watching!