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Whole Organ Tissue Culture

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Abstract

Whole organs can be cultured *ex vivo* using specialized bioreactors, with the goal of repairing or replacing entire organs. This method uses a donor organ that is stripped of all cells, leaving behind the three-dimensional structure, and is then repopulated with new cells. This video demonstrates the whole organ culture of lungs, and shows how a dynamic culture that mimics the mechanical stimulation in the body is needed to induce native tissue properties.

Transcript

In vitro cultures of partial or whole organs are often used in order to accurately model tissue and organ function in various test conditions. Whole organ culture can involve the decellularization, of an excised organ or removal of cells, in order to utilize the native organ structure. This is followed by the recellularization with new cells. The use of specialized bioreactors, are often incorporated into the recellularization process, to mimic tissue growth in the body. This video will introduce the fundamental principals behind whole organ tissue culture, and demonstrate the procedure in the laboratory.

This process begins with harvesting a donor organ. In this example we show a donor lung from a monkey. Through a process called detergent perfusion, the isolated organ is systematically cleansed of its native cell population by a series of washes. Resulting in a sterile acellular organ matrix. Next the tissue matrix is recellularized using specific cell types, such as an established stem cell line. Cells can also be donated by the person who is receiving the engineered tissue. Called autologous cell transplantation. This mitigates rejection and enhances the biocompatibility of the organ. Alternatively, cells can be used from a different donor. Called allogenic transplantation. This may need to be pursued, if a sufficient number of cells cannot be harvested from the potential recipient. Once the cells are seeded on the organ, tissue bioreactors are used to stimulate cell proliferation and direct tissue growth. These reactors aim to dynamically culture the organ and mimic the native environment found in vivo. For example, the organ can be connected to a peristaltic pump in order to simulate blood flow. Now that you've learned about the principals of organ culture, let's take a look at an example procedure involving the whole organ culture of donor lungs.

To begin the donor lungs are placed in a dissecting tray and the pulmonary artery cannulated by introducing a female luer connector into the open cavity. A second female luer connector, is then inserted into the tracheal opening. Phosphate buffered saline or PBS, containing 30 units per milliliters of heparin and five micrograms per milliliter of sodium nitroprusside, is then instilled to facilitate the widening of blood vessels and the removal of trapped air from the lungs. The solution is expelled by natural recoil, and repeated twice more before capping the cannula, to hold the solution in the lungs to dissolve any residual blood. Then both atria are lacerated and the tracheal luer cannula cap is removed, to facilitate fluid drainage. Perfusion is continued with the PBS, heparin and sodium nitroprusside solution, until as much blood as possible is removed from the pulmonary vasculature. To begin decellurization, the lungs are inflated and permeated with deionized water. After five arterial and vascular washes, the lungs are removed from the water and submerged in a detergent called Triton, to remove cells while minimally impacting the organ matrix. The lungs are inflated twice more with Triton solution, before incubating overnight at four degrees Celsius. Following incubation the lungs are washed five more times, with fresh deionized water. Next, the lungs are submerged in 2% Sodium deoxycholate solution and then washed several more times, with water and buffer solution, to facilitate decellularization and removal of cellular debris. When fully cleansed the organ is stored in sterile PBS solution at four degrees Celsius until use.

To mimic the natural behavior of lungs a specialized bioreactor can be used, like the one shown here. First, the main chamber of the reactor is filled with culture medium, that has been equilibrated to the 5% carbon dioxide atmosphere. Then the organ is installed. Once connected the lid is secured and all air is removed, from the tubing using a syringe. The bioreactor is then moved to a tissue culture incubator to equilibrate. Next, the lungs are ventilated with approximately, one full breath every two minutes. And the medium is circulated through the vasculature, via the peristaltic pump, at approximately 10 milliliters per minute, for a total of 30 minutes.

For airway seeding the lungs are inflated, with a cell suspension containing bone marrow derived mesenchymal stem cells. To recellularize the alveoli, which are responsible for the oxygen and carbon dioxide, gas exchange that takes place in the lungs. The lungs are then incubated over night, to allow the cells to attach to the decellularized matrix. After overnight incubation, ventilation is reinitiated, and the cells are allowed to grow in the organ matrix for several days. Next vascular seeding is completed by the gradual introduction of endothelial cells using the peristaltic pump, to initiate the recellularization of small vessels. And then cultured statically for several hours, to facilitate the development of a cellular accurate organ. Culture medium is circulated again, and the cells are cultured for a week to promote growth and attachment under dynamic conditions. Once the tissue growth is complete histology is performed, to confirm the attachment and growth of both the mesenchymal stem cells and endothelial cells, on the vasculature and the airway of the organ. The histology shows the attachment of mesenchymal stem cells and endothelial cells to the alveoli within the matrix scaffold, and small vascular vessels. Creating the appearance of native lung tissue.

Now that you have learned about whole organ culture, let's take a look at some practical applications of this technology outside of the primary focus of regenerative medicine and organ replacement. Whole organ culture can also be used as a way to test pharmaceutical agents or drug delivery devices. For example in this study embryonic mouse thyroids were explanted, cultured, and utilized as an organ model to observe how experimental pharmaceutical agents are transported through the organ and tissue. This simulation may ultimately lead to more realistic representation of how a drug, is transferred within an organ in vivo. Finally, whole organ tissue culture can be used to study, the behavior of tissues under various conditions. For example, intervertebral discs were harvested from bovine tails to study the possible mechanisms, of disc degeneration. Specially designed bioreactors were employed, to induce mechanical loading on the disc, in order to better understand how these loads impact degeneration.

You've just watched Jove's video on Whole Organ Tissue Culture. You should now understand how whole organs, can be cultured in vitro, and how this technique is applied in the bioengineering field. Thanks for watching.