Video Article

October 2014: This Month in JoVE - Visualizing Infectious Disease, Transfecting with the Gene Gun, and a Novel Bioreactor System

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Abstract

Here's a look at what's coming up in the October 2014 issue of JoVE: The Journal of Visualized Experiments.

Right now, the world is witnessing the worst Ebola virus outbreak in history. So this month in JoVE Immunology & Infection, we highlight a technique than can help us better understand how viruses establish infection. Ebola and many other enveloped viruses, including HIV, use glycoprotein spikes on their surfaces to penetrate host cells. Huiskonen et al. use a computational approach to analyze viral envelope spikes, revealing their precise, 3D structure, which is critical for understanding the molecular interactions between certain viruses and their hosts. And this knowledge can guide the design of antiviral drugs and vaccines. Our authors demonstrate the technique with a virus from the family *Bunyaviridae*, but it can be applied to many other viruses that pose biological threats.

Viruses aren't the only pathogens we worry about in JoVE Immunology & Infection. Bacteria and fungi can be just as contagious and deadly. Every year the fungus *Cryptococcus neoformans* infects more than 1 million people worldwide and causes over 600,000 deaths mostly in sub-Saharan Africa, where it is one of the leading causes of death in people with AIDS. Cryptococcal cells are generally killed by macrophage cells of the immune system; but some can survive latently in macrophages, then later release into the surroundings. This phenomenon is poorly understood, so Stukes and Casadevall have developed a way to infect macrophages with Cryptococcus *in vitro* and observe them over an extended period using time-lapsed microscopy. This is a promising technique for studying the complex interactions between *Cryptococcus* and host macrophages, and can be applied to other fungal pathogens as well.

In JoVE Neuroscience, we feature a technique that combines ballistics, or the science of shooting, with genetic engineering. Bioballistic gene guns were originally developed to inject genetic information into plant cells. Arsenault et al. have applied this technique to mammalian tissues. Where terminally differentiated cells, like neurons, can difficult to transfect using conventional methods. By using an improved and patented bioballistic delivery method, our authors quickly and efficiently transfect various genes into distinct tissue regions.

A common goal in JoVE Bioengineering is to culture bacteria or eukaryotic cells in large scales for industrial or biomedical applications. Some cultures, like bacteria and yeasts, can be grown in suspension, But many mammalian cells grow in aggregates, while others grow anchored to a substrate Obom et al. present a novel bioreactor system that can support all three types of cultures. They focus on anchorage-dependent cells, which have culture parameters that can be difficult to control, especially in large scales. Small beads called microcarriers, are added to the culture, to increase the surface area for cell growth, allowing anchorage-dependent cells to be grown in suspension. This system has tremendous potential for growing a variety of cell types and products for many applications.

You've just had a sneak peek of the October 2014 issue of JoVE. Visit the website to see the full-length articles, plus many more, in JoVE: The Journal of Visualized Experiments.

Video Link

The video component of this article can be found at http://www.jove.com/video/5518/

Protocol

Cultivation of Mammalian Cells Using a Single-use Pneumatic Bioreactor System

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Using a pneumatic bioreactor, we demonstrate the assembly, operation, and performance of this single-use bioreactor system for the growth of mammalian cells.

Visualizing Non-lytic Exocytosis of *Cryptococcus neoformans* from Macrophages Using Digital Light Microscopy

Sabriya Stukes, Arturo Casadevall

Department of Microbiology and Immunology, **Albert Einstein College of Medicine**

We describe how to visualize macrophage-*C. neoformans* (Cn) interactions in real time, with specific emphasis on the process of non-lytic exocytosis using digital light microscopy. Using this technique individually infected macrophages can be studied to ascertain various aspects of this phenomenon.

Averaging of Viral Envelope Glycoprotein Spikes from Electron Cryotomography Reconstructions using Jsubtomo

Juha T. Huiskonen, Marie-Laure Parsy, Sai Li, David Bitto, Max Renner, Thomas A. Bowden

Oxford Particle Imaging Centre, Division of Structural Biology, Wellcome Trust Centre for Human Genetics, **University of Oxford**

An approach is presented for determining structures of viral membrane glycoprotein complexes using a combination of electron cryo-tomography and sub-tomogram averaging with the computational package Jsubtomo.

Regioselective Biolistic Targeting in Organotypic Brain Slices Using a Modified Gene Gun

Jason Arsenault[1,2], Andras Nagy[1], Jeffrey T. Henderson[1], John A. O'Brien[2]

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Recent improvements in organotypic brain slice preparations have permitted their exploitation for biotechnological applications. Organotypic slices maintain local structural characteristics of *in vivo* biology, including functional synaptic connections. Here we present a regioselective biolistic delivery method to label and genetically manipulate these slices.

Disclosures

No conflicts of interest declared.