Video Article

October 2012: This Month in JoVE

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Abstract

Here are some highlights from the October 2012 issue of Journal of Visualized Experiments (JoVE).

Grønlund *et al.* demonstrate how to isolate and analyze specific cell types in plant leaves expressing green fluorescent protein (GFP) using fluorescence-activated cell sorting (FACS). This method overcomes the interfering fluorescence of the chlorophyll found in leaves, and distinguishes GFP-expressing protoplasts from non-GFP protoplasts.

In JoVE Neuroscience, Babona-Pilipos et al. demonstrate how to construct a chamber for measuring galvanotaxis, or cell migration within an electric field. Through time-lapse imaging and image analysis, the authors can study the migratory behavior of neural precursor cells in an electric field, which may lead to the use of electrical stimulation to direct neural precursors to sites of injury or disease.

Articles involving microfluidic platforms have a significant publication history in JoVE. Harris et al. have released three articles, which involve a microfluidic device for separating axons from neuronal cell bodies. This month in JoVE Neuroscience, Higashimori et al. use this microfluidic platform to investigate the interactions between neuronal axons and glial cells, which are critical for the physiological function of the nervous system.

In JoVE Bioengineering, two research groups demonstrate the novel bioadhesive properties of chitosan-a polymer derived from chitin, which is found in fungal cell walls or in the exoskeletons of crustaceans and insects. Chitosan is used in many industrial and agricultural applications, and bioengineers are also finding uses for it in surgical applications. Foster et al. have developed a laser-activated surgical film called Surgilux, which combines chitosan with indocyanine green ICG), a photosensitive dye. This surgical film binds strongly to tissue, such as muscle, after laser irradiation. Lauto et al. developed a surgical film that combines chitosan with the photoactive dye, rose Bengal. This novel film also binds strongly to tissues, such as intestine, after it is irradiated. These adhesive films are biocompatible and may potentially be used in various surgical procedures in place of sutures.

In JoVE Clinical and Translational Medicine, Fiema et al. demonstrate a high-throughput technique for validating biomarkers in graft vs. host disease, a common and life-threatening complication of cell or tissue transplantation. The authors use commercially available ELISAs to analyze multiple proteins in sequential fashion.

In JoVE Immunology and Infection, Keyel *et al.* demonstrate how to measure the real-time kinetics of immune cell responses to bacterial toxins using high-speed live cell microscopy. This method can be used to show how immune cells respond to bacterial toxins. Combined with high-speed 3D confocal microscopy, this technique can also visualize the cellular repair response.

In JoVE Applied Physics, Borisenko et al. determine the electronic structure of complex materials using angle-resolved photoemission spectroscopy in a synchotron radiation facility. By combining recent advances in synchotron radiation, surface science, and cryogenics, this method can gain a precise picture of the energy and momentum of electrons inside a solid, and address key questions in the field of condensed matter physics.

This preview summarizes just a few of the notable video-articles available in the October 2012 issue of JoVE. For additional videos, please visit www.jove.com.

Video Link

The video component of this article can be found at https://www.jove.com/video/5025/

Protocol

A Chitosan Based, Laser Activated Thin Film Surgical Adhesive, 'SurgiLux': Preparation and Demonstration.

L. John R. Foster, Elizabeth Karsten

Bio/Polymer Research Group, University of New South Wales

The fabrication of a novel, flexible thin film surgical adhesive from FDA approved ingredients, chitosan and indocyanine green is described. Bonding of this adhesive to collagenous tissue through a simple activation process with a low-powered infra-red laser is demonstrated.

Fabrication and Application of Rose Bengal-chitosan Films in Laser Tissue Repair

Antonio Lauto[1], Marcus Stoodley[2], Matthew Barton[1], John W. Morley[1], David A. Mahns[1], Leonardo Longo[3], Damia Mawad[1]
[1]Bioelectronics and Neuroscience (BENS) research group, University of Western Sydney, NSW Australia, [2]Australian School of Advanced Medicine, Macquarie University, NSW Australia, [3]School of Medicine, University of Siena, Italy

Sutures are usually needed to repair tissue during surgical procedures. However, their application can be problematic as they are invasive and may damage tissue. The fabrication and application methods of a novel tissue adhesive are here reported. This adhesive film is laser-activated and does not require the use of sutures.

A Galvanotaxis Assay for Analysis of Neural Precursor Cell Migration Kinetics in an Externally Applied Direct Current Electric Field

Robart Babona-Pilipos[1], Milos R. Popovic[2], Cindi M. Morshead[3]
[1]Institute for Biomaterials and Biomedical Engineering, University of Toronto, [2]Lyndhurst Centre, Toronto Rehabilitation Institute, [3]Department of Surgery, University of Toronto

In this protocol we demonstrate how to construct custom chambers that permit the application of a direct current electric field to enable time-lapse imaging of adult brain derived neural precursor cell translocation during galvanotaxis.

Cell specific analysis of Arabidopsis leaves using fluorescence activated cell sorting

Jesper T. Grønlund[1], Alison Eyres[1], Sanjeev Kumar[1], Vicky Buchanan-Wollaston[1, 2], Miriam L. Gifford[1, 2]
[1]School of Life Sciences, University of Warwick , [2]Warwick Systems Biology, University of Warwick

A method for producing *Arabidopsis* leaf protoplasts that are compatible with fluorescence activated cell sorting (FACS), allowing for studies of specific cell populations. This method is compatible with any *Arabidopsis* line that expresses GFP in a subset of cells.

Visualization of Bacterial Toxin Induced Responses Using Live Cell Fluorescence Microscopy

Peter A. Keyel[1], Michelle E. Heid[1], Simon C. Watkins[2], Russell D. Salter[1]
[1]Department of Immunology, University of Pittsburgh School of Medicine, [2]Department of Cell Biology and Physiology, University of Pittsburgh School of Medicine

Methods for purifying the cholesterol binding toxin streptolysin O from recombinant *E. coli* and visualization of toxin binding to live eukaryotic cells are described. Localized delivery of toxin induces rapid and complex changes in targeted cells revealing novel aspects of toxin biology.

High Throughput Sequential ELISA for Validation of Biomarkers of Acute Graft-Versus-Host Disease

Bryan Fiema[*], Andrew C. Harris[*], Aurelie Gomez, Praechompoo Pongtornpipat, Kelly Lamiman, Mark T. Vander Lugt, Sophie Paczesny
Pediatric Blood and Marrow Transplant Program, University of Michigan
[*] These authors contributed equally

High throughput validation of multiple candidate biomarkers can be performed by sequential ELISA in order to minimize freeze/thaw cycles and use of precious plasma samples. Here, we demonstrate how to sequentially perform ELISAs for six different validated plasma biomarkers[1-3] of graft-versus-host disease (GVHD)[4] on the same plasma sample.

Imaging Analysis of Neuron to Glia Interaction in Microfluidic Culture Platform (MCP)-Based Neuronal Axon and Glia Co-culture System

Haruki Higashimori[1], Yongjie Yang[1, 2]
[1]Department of Neuroscience, Tufts University, [2]Neuroscience Program, Tufts Sackler School of Graduate Biomedical Sciences

This study describes the procedures of setting up a novel neuronal axon and (astro)glia co-culture platform. In this co-culture system, manipulation of direct interaction between a single axon (and single glial cell) becomes feasible, allowing mechanistic analysis of the mutual neuron to glial signaling.

Angle-resolved Photoemission Spectroscopy At Ultra-low Temperatures

Sergey V. Borisenko[1], Volodymyr B. Zabolotnyy[1], Alexander A. Kordyuk[1, 2], Danil V. Evtushinsky[1], Timur K. Kim[1, 3], Emanuela Carleschi[4], Bryan P. Doyle[4], Rosalba Fittipaldi[5], Mario Cuoco[5], Antonio Vecchione[5], Helmut Berger[6]
[1]Institute for Solid State Research, IFW-Dresden, [2]Institute of Metal Physics of National Academy of Sciences of Ukraine, [3]Diamond Light Source LTD, [4]Department of Physics, University of Johannesburg, [5]CNR-SPIN, and Dipartimento di Fisica "E. R. Caianiello", Università di Salerno, [6]Institute of Physics of Complex Matter, École Polytechnique Fédérale de Lausanne

The overall goal of this method is to determine the low-energy electronic structure of solids at ultra-low temperatures using Angle-Resolved Photoemission Spectroscopy with synchrotron radiation.

Fabrication of a Microfluidic Device for the Compartmentalization of Neuron Soma and Axons

Joseph Harris[1], Hyuna Lee[1], Behrad Vahidi[1], Christina Tu[2], David Cribbs[3], Noo Li Jeon[1], Carl Cotman[3]
[1]Department of Biomedical Engineering, University of California, Irvine, [2]Stem Cell Research Center, University of California, Irvine, [3]Institute for Brain Aging and Dementia, University of California, Irvine

In this video we demonstrate the technique of soft lithography with polydimethyl siloxane (PDMS) which we use to farbricate a microfluidic device for culturing neurons.

Preparing E18 Cortical Rat Neurons for Compartmentalization in a Microfluidic Device

Joseph Harris[1], Hyuna Lee[1], Christina Tu Tu[2], David Cribbs[3], Carl Cotman[3], Noo Li Jeon[1]
[1]Department of Biomedical Engineering, University of California, Irvine, [2]Stem Cell Research Center, University of California, Irvine, [3]Institute for Brain Aging and Dementia, University of California, Irvine

In this video we demonstrate the preparation of E18 Cortical Rat Neurons.

Non-plasma Bonding of PDMS for Inexpensive Fabrication of Microfluidic Devices

Joseph Harris[1], Hyuna Lee[1], Behrad Vahidi[1], Cristina Tu[2], David Cribbs[3], Carl Cotman[3], Noo Li Jeon[1]
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In this video we demonstrate how to use the neuron microfluidic device without plasma bonding.

Disclosures

No conflicts of interest declared.

