Video Article

# April 2014: This Month in JoVE - Bioengineering for ACL Tears, Neuroscience behind Insect Locomotion, Improved Genetic Engineering of Crops, and Synthesizing Secret Ink

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## Abstract

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

In the Bioengineering section we have a video article that examines the anterior cruciate ligament (ACL), which is critical for keeping the knee stable when pivoting or planting. Torn or ruptured ACLs are among the most common of all serious knee injuries, especially in athletes, and even partial tears can hurt knee stability and function. Because they can't heal on their own, torn ACLs are often treated surgically, sometimes by reconstructing the ligament with a tendon grafted from the patient's own body or from a cadaver. Every year, over 100,000 ACL reconstructions are performed in the United States. The torn ligaments are usually discarded as surgical waste, but Gupta *et al.* see them as a valuable source of cells for potentially repairing ACLs. They developed a method for harvesting and expanding human ACL-derived cells then growing them on scaffolds to form tissue-engineered patches. Under scanning electron microscopy, the engineered matrix demonstrates good cellular adherence and growth. This technique could lead to methods for strengthening damaged ligaments and enhancing ACL repair, especially for those wishing to maintain an athletic lifestyle.

In the Neuroscience section we examine motor control in insects, which are among the most athletic creatures on Earth. They are especially good at adapting to sudden changes in terrain, so scientists are interested in correlating neural activity with behavior in insects. Guo *et al.* have developed a method for recording brain activity in the discoid cockroach, also known as the tropical cockroach, as they explore an arena or as they encounter obstacles that cause them to change course. The authors also demonstrate how to collect the data for subsequent analysis, which may yield insight into the neural control of behavior.

In JoVE Environment we feature a technique for transient gene expression in plants. To express gene products in specific parts of the cell, plants often have several copies of a gene and express them separately in different organelles (such as the chloroplast and peroxisome) or in the cytosol. Therefore, genetic engineers may have to target multiple duplicated genes to manipulate expression in different cell parts. To get around this problem, Mattozzi *et al.* use a novel method for multiple-organelle targeting that takes advantage of alternatively spliced mRNAs, which are sent to different locations in the cell. The targeting constructs are designed using Gibson assembly, a one-step cloning method, and then delivered using a gene gun. This technique offers great flexibility in terms of genetic design, and can be applied to large-scale selective genetic engineering in agriculture or the environment.

In the Chemistry section we feature an article that uses modern chemical techniques to examine two bright blue pigments that were synthesized in ancient societies. Calcium copper tetrasilicate, commonly known as Egyptian blue, is thought to be the first synthetic pigment and used in Egypt as early the 4th Dynasty (ca. 2500 BC), the height of the pyramid-building era. Barium copper tetrasilicate, also known as Han blue, was later developed in China during the beginning of the Zhou Dynasty, or the height of the bronze age (1045-771 BC). Nowadays, scientists are interested in the strong near infrared (NIR) emission properties of these two pigments, which can be utilized for optical sensors. Related to this application is the ability to exfoliate copper tetrasilicates into 2D monolayers, or nanosheets. Johnson-McDaniel and Salguero demonstrate how to synthesize these two copper tetrasilicate pigments and exfoliate them into monolayer nanosheets. Egyptian blue spontaneously exfoliates into monolayers when stirred in hot water, whereas Han blue requires ultrasonication in organic solvents. Our authors show that both the bulk and nanosheet forms of the two pigments are strong near infrared emitters with potential applications ranging from security inks to biomedical imaging.

You've just had a sneak peek of the April 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 https://www.jove.com/video/5323/

## Protocol

### Transient Gene Expression in Tobacco using Gibson Assembly and the Gene Gun

Matthew d. Mattozzi[1,2], Mathias J. Voges[1,2,3], Pamela A. Silver[1,2], Jeffrey C. Way[1,2]

[1]Synthetic Biology Platform, Wyss Institute for Biologically Inspired Engineering, **Harvard University**, [2]Department of Systems Biology, **Harvard Medical School**, [3]Department of Biotechnology, **Delft University of Technology**

This work describes a novel method for selectively targeting subcellular organelles in plants, assayed using the BioRad Gene Gun.

### Surgical Retrieval, Isolation and *in vitro* Expansion of Human Anterior Cruciate Ligament-derived Cells for Tissue Engineering Applications

Ashim Gupta[1], Kevin Sharif[2], Megan Walters[2], Mia D. Woods[1], Anish Potty[2], Benjamin J. Main[4], Saadiq F. El-Amin III[1,2,3]

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For future applications as a patch to repair partial tears of the Anterior Cruciate Ligament (ACL), human ACL derived cells were isolated from tissue obtained during reconstructive procedures, expanded *in vitro* and grown on tissue engineered scaffolds. Cellular adhesion and morphology was then performed to confirm biocompatibility on scaffold surface.

### Exfoliation of Egyptian Blue and Han Blue, Two Alkali Earth Copper Silicate-based Pigments

Darrah Johnson-McDaniel, Tina T. Salguero

Department of Chemistry, **The University of Georgia**

The preparation and exfoliation of $CaCuSi_4O_{10}$ and $BaCuSi_4O_{10}$ are described. Upon stirring in hot water, $CaCuSi_4O_{10}$ spontaneously exfoliates into monolayers, whereas $BaCuSi_4O_{10}$ requires ultrasonication in organic solvents. NIR imaging illustrates the NIR emission properties of these materials, and aqueous dispersions of these nanomaterials are useful for solution processing.

### Extracellular Wire Tetrode Recording in Brain of Freely Walking Insects

Peiyuan Guo, Alan J. Pollack, Adrienn G. Varga, Joshua P. Martin, Roy E. Ritzmann

Department of Biology, **Case Western Reserve University**

We previously developed a technique for implanting tetrode wires into the central complex of cockroach brains that allows us to monitor activity in individual units of tethered cockroaches. Here we present a modified version of that technique that allows us to also record brain activity in freely moving insects.

## Disclosures

No conflicts of interest declared.

 