Bio Focus

Nonlinear optical microscopy enables noninvasive quality control of tissue-engineered devices

Tissue engineering integrates engineered and biological components to create medical devices that can regenerate damaged tissue and improve wound healing. As part of the growing field of regenerative medicine, tissue-engineered constructs allow for patient-specific approaches to treatment. Instead of waiting years for a transplant, patients could receive implanted tissues designed for their individual condition.

However, regulation of these constructs before implantation remains an obstacle to their widespread use. Because the devices are surgically implanted, an effective quality control must provide reliable measurements of each construct's likelihood to succeed in the patient, without disturbing the tissue.

A team of researchers from the University of Michigan has proposed a new method for assessing the viability of individual constructs prior to implantation. Their results, published in the May 20 online edition of *Biomaterials* (DOI: 10.1016/j.biomaterials.2014.04.080), suggest that label-free nonlinear optical molecular microscopy (a technique that uses near-infrared light to penetrate through layers of living tissue) could be used to quickly and quantitatively assess the viability of constructs while leaving them intact.

"It's fundamentally a very technically challenging project," said lead researcher Mary-Ann Mycek, a professor of biomedical engineering at the University of Michigan. "While optical technology is widely used in materials research, we're now talking about taking a biomaterial and characterizing cellular viability from inside a living tissue."

Traditional methods of tissue evaluation are invasive—such as taking biopsies or using external fluorescent proteins—or imprecise—such as the glucose consumption test.

Instead, the researchers harnessed the metabolic cofactors NAD(P)H and FAD—enzymes naturally occurring in the tissue that fluoresce when excited by light—in *Ex Vivo* Produced Oral Mucosa Equivalent constructs, or EVPOMEs, developed by Stephen Feinberg and Cynthia Marcelo. "Measuring the relative ratios of the fluorescence coming from those cells tells us about their metabolism," said Mycek, where this allows the team to noninvasively assess the constructs' function.

The researchers analyzed both less viable thermally stressed EVPOMEs and healthy controls to determine whether optical imaging techniques could distinguish between the two. Nonlinear microscopy, which uses near-infared light, can penetrate the surface of the tissue and measure activity underneath from many different channels of information, optically sectioning tissue into 1 µm layers. The optical method was found to noninvasively evaluate EVPOME viability as successfully as traditional destructive assays.

Notably, Mycek and her colleagues used oral mucosal tissue constructs made from primary human cells that had been approved by the FDA in Phase I and Phase II clinical trials that are being carried out by Feinberg, an oral and maxillofacial surgeon at the University of Michigan. "This study intentionally introduces the kind of patient-to-patient variability that you'd expect in a clinical setting," said Mycek, making their findings more relevant to real-world applications.

"Advanced optical microscopy techniques such as those used here have the potential to characterize tissue-engineered constructs in ways that were not possible before," said Maryann Fitzmaurice, a senior research associate at Case Western Reserve University. "This imaging technology will be key to widespread clinical adoption of engineered tissues, as it will enable timely and accurate assessment of the quality, safety, and efficacy of these important medical devices."

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