Scaffold Structure and Cell Function Through Multimodal Imaging and Quantitative Visualization

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Tissue engineered medical products (TEMPs) are often three-dimensional (3D) hybrid materials consisting of a porous scaffold upon which the tissue is grown. While it is generally understood that a complex interaction of many variables influences the success of TEMPs, the precise nature of these interactions has yet to be worked out in many instances. A significant difficulty in furthering the understanding of the interaction between these factors and cell behavior is the lack of a high-resolution imaging technique that can penetrate deeply and nondestructively into the scaffold. An approach that uses advanced optical imaging to noninvasively monitor the developing tissue was developed. However, before any assessment of the tissue viability can be made, the volumes of imaging data must be rigorously analyzed. Therefore, an equally important component of this effort is image visualization and quantification. Progress in these areas is summarized below.

MULTIMODAL IMAGING

An instrument that can gather information on a TEMP using multiple imaging modalities was constructed. This means that each channel of imaging data provides different but complimentary information. Optical coherence microscopy (OCM) was chosen as the technique to image scaffold, cell, and tissue structure because of its unique combination of high resolution (^{a}1 μm) and high sensitivity (> 100 dB). OCM is an interferometric technique that uses both confocal and coherence gating mechanisms for stray light rejection, rendering it comparable in resolution to laser scanning confocal microscopy but far superior in imaging depth. Confocal fluorescence microscopy (CFM) was added to the

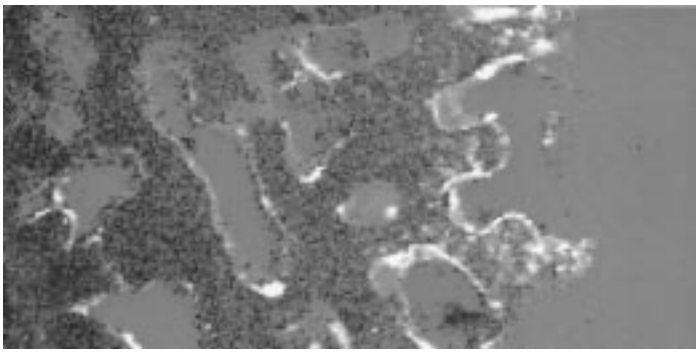

Figure 1: Image of merged and registered OCM and CFM images of the cultured PCL scaffold at 145 _m below the surface.

OCM to collect information on cell function using traditional cell-staining techniques. In the collinear instrument, volumetric images of cell and scaffold structure were collected using the OCM channel and cell function using the CFM channel. Each channel is then overlayed in the rendered image for maximal insight. Figure 1 displays merged and registered OCM and CFM images 145 μm below the surface of a TEMP. The TEMP consists of a volume fraction of 50 percent poly(ε-caprolactone) (PCL) scaffold that was cultured with fetal chick osteoblasts for 10 weeks and stained with a nuclear stain. In Figure 1, the regions of low OCM signal are red (pores), high OCM signal are black (scaffold), and regions of high CFM signal are yellow (cells). CFM complements OCM by allowing us to positively identify stained tissues at more shallow depths. Once identified, OCM allows us to discriminate these tissues from scaffold, and thus view them at a greater depth. This will form the basis of structure-property relationships for TEMPs based on microscopic characterization of scaffold properties and concomitant cellular responses.

QUANTITATIVE VISUALIZATION

Quantification of scaffold properties must be performed to establish scaffold structure and cell function relationships and to optimize scaffold design. One goal is to design an approach that is valid with any pore structure. Figure 2 displays a volumetric view of the PCL scaffold images collected using X-ray computed tomography. In this figure, the pores are colored in red and the scaffold in green.

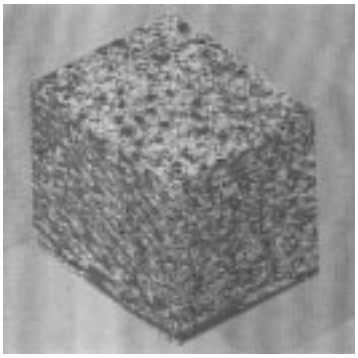

Figure 2: X-ray computed tomographic reconstruction of a PCL based scaffold. Dimensions: 1.0 mm on each side.

From viewing this image, one gets a qualitative sense of the need for such an approach. Heterogeneity in the microstructure is exhibited by the difference in pore size, shape and anisotropy as seen on the different faces of the volume. Pore volume, size distribution, tortuosity, and connectivity are metrics of interest for the scaffold microstructure. An example of the type of information gleaned from the imaging data is shown on page 20. Figure 3 displays the pore size as measured by the chord length distribution function (CLDF). The CLDF is the probability of finding a chord of length l between x and x + dx entirely in one phase. Chords are defined as the segments formed by the intersection of lines with the interface between two phases.

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Scaffold Structure...

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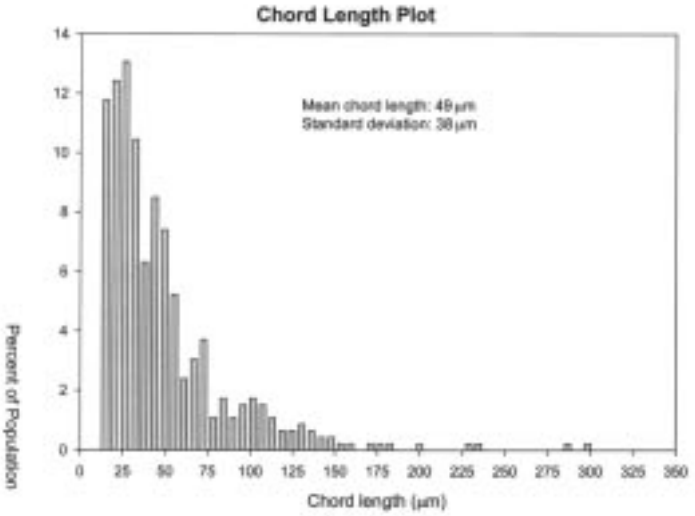

Figure 3: Example of a CLDF from one image plane in Figure 2.

Another important aspect of the effort to optimize scaffold design is the need to balance competing requirements. High porosity is required because of the need for cell migration, proliferation, and nutrient influx. However, the drive towards higher and higher pore volumes opposes the need for certain mechanical property requirements, especially in orthopedic applications. To this end, we are using 3D finite element analysis (FEA) to develop an analytical tool to predict the relationship between the effective properties and individual constituent properties of TEMPs based on real material images. This relationship, plus the analysis of the structural problem of interest, provides a means of optimizing the performance of

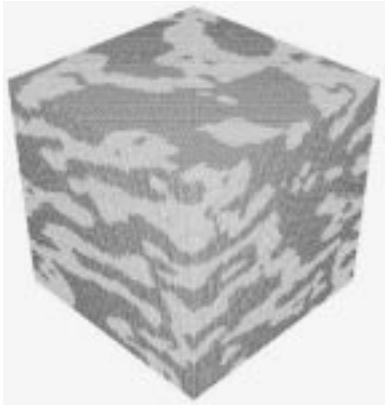

Figure 4: Finite element mesh of a subsection of scaffold shown in Figure 2. The dimension is 274 μm on each side.

TEMPs by varying individual constituent properties without conducting a variety of time-consuming experiments. Initially, the properties of interest are anisotropic elastic constants. Figure 4 displays a typical mesh of FEA based on a section of the PCL scaffold from Figure 2.

This work represents a systematic, integrated approach to the study of structure/function relationships and optimal design in TEMPs. Extracted metrics for the anisotropic scaffold microstructure and properties can be used to understand their influence on cell function, and on a larger scale, TEMP viability.

For more information on this topic, contact falandis@nist.gov or mchiang@nist.gov (Polymers Division, NIST). For information involving chemical imaging of TEMPs, contact marcus.cicerone@nist.gov and see the project page titled, "Coherent Anti-Stokes Raman Micro-spectroscopy (μ-CARS) for Understanding Tissue Growth in Scaffold Constructs," by M. Cicerone and T. Kee, given in http://polymers.nist.gov/annuals/2003/polymers2003.pdf.

Bio**Ink**

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Centerpulse's largest single shareholder with 18.3 percent of the equity capital. The deal to create the world's biggest orthopedics group ended October 2. U.S.-based Zimmer's bid trumped an agreed offer for Centerpulse by Britain's **Smith & Nephew** Plc.

Roche has launched the AmpliChip CYP450 microarray, the company's first microarray for clinical applications, in the United States. The product enables clinical diagnostic laboratories to identify certain naturally occurring variations (called polymorphisms) in two genes, the CYP2D6 and CYP2C19, which play a major role in drug metabolism. These variations affect the rate at which an individual metabolizes many drugs used to treat cardiovascular disease, high blood pressure, depression, attention-deficit hyperactivity disorder, and more. Knowledge of these variations, when considered with other contributing factors, can help a physician select the best drug and set the right dose for a patient sooner, as well as avoid drugs that may cause the patient to suffer serious adverse reactions. Roche expects the AmpliChip CYP450 microarray-based assay to generate annual revenues of more than $100 million by 2008.