Micro-CT Imaging

Tissue engineering has immense potential to aid in the functional restoration of damaged or degenerated tissues and organs. Yet, as tissue-engineered constructs become more sophisticated, critical design trade-offs must be made in the selection and integration of cell and scaffold parameters.

For example, the scaffold material and architecture that provide optimal mechanical properties may not be ideally suited for delivering cells and proteins or for facilitating the invasion of host cells and vasculature from surrounding tissue. Similarly, whereas the ability to direct formation of a suitable type of extracellular matrix within a defect is critically important, long-term restoration of function also is highly dependent on the quantity and quality of tissue formed.

To address these design decisions, clinically relevant models and quantitative outcome metrics are needed to evaluate, benchmark, and optimize tissue-engineering technologies. Nondestructive imaging techniques are increasingly providing a powerful set of quantitative tools to provide such information and thereby aid in the development and evaluation of new approaches to engineering tissues and organs.

Several micro-imaging techniques are now available to biomedical researchers, which include magnetic resonance microscopy, positron emission tomography, ultrasound microscopy, and x-ray micro-computed tomography (micro-CT). Each of these techniques has advantages and disadvantages and provides information that is typically complementary to that of the other approaches.

Magnetic resonance imaging, for example, provides excellent soft tissue contrast and has been used to noninvasively monitor cellular bioenergetics within microencapsulated constructs in vitro and in vivo. It also allows evaluation of the effects of different seeding methods on cell distribution within 3D constructs.

High-resolution x-ray micro-CT was first developed in the early 1980s and has been applied widely in material science, bioengineering, and, more recently, tissue engineering. Micro-CT combines micro-focal spot x-ray projections rotated through multiple viewing directions to provide 3D reconstructed images of samples at voxel resolutions of 6 to 50 μm, over a field of view of approximately 50 mm.

Micro-CT images represent spatial distributions of linear attenuation coefficients determined by the energy of the x-ray source and the atomic composition of the material sample. Local density distributions can be determined by calibrating with a material of known density. Since the imaging process is nondestructive, the internal features of a sample may be examined repeatedly over time.

A wide range of materials may be examined directly with micro-CT, including ceramics, polymers, and mineralized tissues. Micro-CT imaging can also be extended to soft tissues such as blood vessels that have been infiltrated or perfused with a contrast agent.

Whereas early micro-CT systems were custom-built and filled an entire room, commercial tabletop systems are now available. The newest generation of systems, which use multiple fan beams simultaneously to reduce scanning time and x-ray exposure, allow for in vivo imaging and therefore longitudinal data collection.

Micro-CT images provide a basis for quantitative evaluation of biomaterial and tissue samples. After reconstructed images are thresholded, stereologic algorithms are applied to quantify volume fraction as well as parameters related to the microstructure and orientation of the imaged samples.

In biopsies from osteoporotic patients, for example, micro-CT has been used to measure thinning and fenestration of trabecular struts within cancellous bone. More recent applications have included analysis of biomaterial scaffolds, quantification of mineralized matrix formation within bone repair constructs in vitro and in vivo, and 3D imaging of angiogenic responses within polymer implants.

Microarchitecture has a substantial influence on the mechanical and biological properties of porous biomaterial scaffolds. The mechanical stiffness and strength of a scaffold along a specific axis depend not only on volume fraction or porosity but also the alignment of material within the scaffold. Porosity, pore size, and pore interconnectivity also strongly influence mass transport within scaffolds and therefore the biological responses after cell-seeding in vitro or implantation in vivo.

Because cells require surfaces for adhesion, the surface-to-volume ratio of scaffolds may be an important variable affecting cell-seeding

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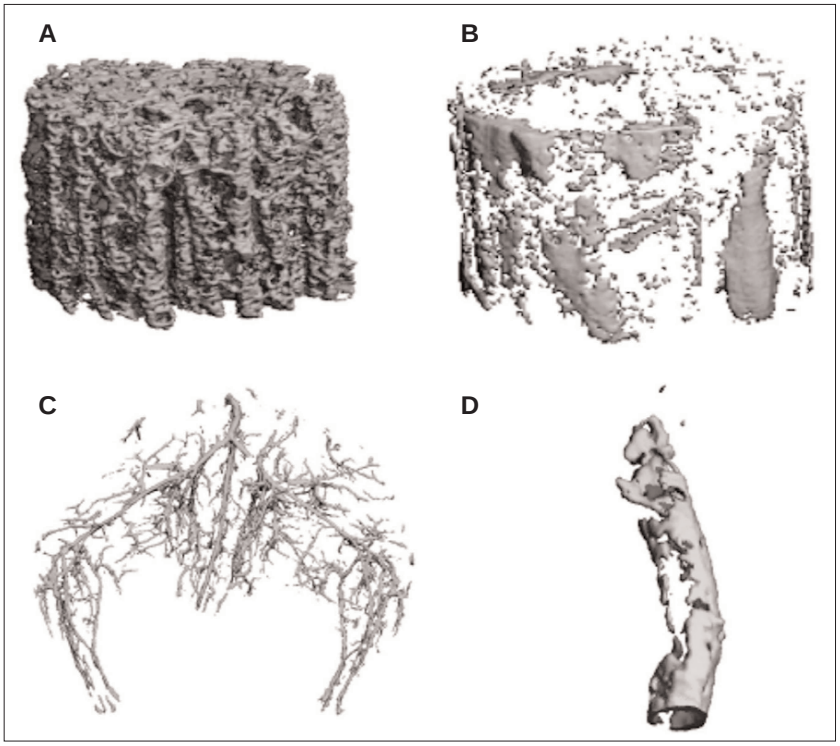

Micro-CT provides high-resolution 3D images of a variety of tissues and biomaterials. ***A,*** By setting a low threshold, micro-CT can be used to analyze polymer scaffolds, such as this 5-mm-diameter poly(L-lactide-co-DL-lactide) (PLDL) scaffold with random microporosity and axially oriented macroporosity. ***B,*** At higher thresholds, mineralized matrix synthesis by marrow-derived stem cells seeded within PLDL scaffolds and exposed to osteoblastic differentiation media may be measured over time in culture.

C, Soft tissue images , such as mouse hind-limb vasculature, may be obtained by combining micro-CT with a silicon-based contrast agent containing lead chromate. ***D,*** Vascular calcification can also be investigated by micro-CT, as shown by the mineral deposition within the aorta of a 2-week-old genetically engineered mouse.

efficiency and subsequent matrix synthesis. Yet, few scaffold structure-function studies have been completed due partly to the difficulty of quantifying microarchitectural parameters.

Micro-CT imaging and analysis allows measurement of the morphology and microstructural orientation (anisotropy) of biomaterial scaffolds produced with a wide variety of methods. For example, porous poly(L-lactide-co-DL-lactide) (PLDL) scaffolds may be created using a solution coating and porogen decomposition technique.

Briefly, the method involves coating 50- to 100-μm wires with polymer mixed with varying concentrations of a porogen agent that decomposes violently at elevated temperatures to create random microporosity with high surface area for cellular attachment. Following decomposition and quenching, the wires are removed to reveal axially oriented macroporosity, which provides more efficient mass transport throughout the scaffold.

Using micro-CT, the effects of porogen concentration and other manufacturing variables on polymer scaffold porosity, average pore size and internal strut thickness, pore orientation, and surface-to-volume ratio can be evaluated.

By inverting the 3D images and labeling distinct volume entities, it is also possible to assess whether the pore space within the polymer scaffolds is fully connected. The porosity of PLDL scaffolds, for example, was found to be over 99% interconnected. Scaffold interconnectivity is important because isolated pore spaces may slow cellular and vascular infiltration.

When combined with subsequent mechanical testing and in vitro or in vivo test bed studies, micro-CT analysis allows scaffold microstructure-function relationships to be quantified. It thereby provides a rationale basis for optimization of scaffold design for specific tissue-engineering applications.

Micro-CT imaging also may be combined with finite element modeling or rapid prototyping methods, such as stereolithography, to provide computer-

aided tissue engineering. High-resolution images of natural tissue morphology may be converted to models for local stress analysis or for input to stereolithography systems.

Rapid prototyping systems have been adapted recently to improve resolution and incorporate the use of biocompatible polymers and ceramics. Using this image-based manufacturing approach, bioresorbable polymer scaffolds have been developed that match the shape of a bone defect or mimic the complex 3D morphology of a heart valve. In addition to defining the scaffold external geometry, this method also allows the internal microarchitecture of the scaffold to be defined precisely.

A variety of in vitro and in vivo models of mineralization are available to test bone repair constructs. Micro-CT provides an efficient, nondestructive tool to quantitatively measure the amount and distribution of mineralized matrix formation throughout 3D constructs. Following micro-CT scanning, samples are still intact for biomechanical, histologic, or biochemical evaluation.

Quantitative outcome measures such as these provide the ability to optimize bone repair construct parameters and benchmark competing technologies. For example, by using micro-CT analysis, the relative ability of mesenchymal stem cell-based therapies to regenerate bone for spine fusion or long-bone segmental defects may be compared to osteoinductive protein, gene delivery, or combination therapies.

If sterile conditions are maintained, cell-seeded constructs may be scanned repeatedly to monitor mineral formation within 3D constructs in vitro over time. Even more exciting is the potential to collect quantitative longitudinal data on mineralization within implanted bone repair constructs using in vivo micro-CT systems.

Unlike mineralized tissues, soft tissues such as blood vessels or heart valves do not typically provide sufficient x-ray attenuation for micro-CT imaging. However ectopic mineralization of vascular tissues is common with aging or in pathologic conditions and may represent a failure of tissue-engineered implants. Micro-CT may be used to measure the amount and distribution of mineral deposition within vascular tissues and constructs.

Micro-CT imaging of vascular tissues in the absence of mineralization is also possible if the tissues are filled or surrounded with a contrast agent, such as barium sulfate or lead chromate, which have an atomic number greater than the surrounding tissue. This approach has been used to produce 3D images of the vasculature within a variety of organs at a nominal resolution of 15 to 30 μm.

One advantage of this approach over 2D histologic methods is that the 3D connectivity of the vascular network may be investigated. Of greater relevance to tissue engineering, micro-CT imaging combined with a perfused contrast agent may be used to measure angiogenic responses following vascular injury or within subcutaneously implanted polymer sponges. Novel contrast agents are being developed that may extend micro-CT vascular imaging to in vivo studies.

Quantitative tools such as micro-CT are needed to push tissue engineering beyond a qualitative, observational field and to accelerate the clinical realization of these technologies. As faster, higher resolution micro-CT systems become available for both in vitro and in vivo studies and the development of improved contrast agents allows micro-CT imaging to be extended to nonmineralized tissues, additional novel applications are sure to emerge.

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