J Musculoskel Neuron Interact 2004; 4(4):399-400

Perspective Article

Hylonome

Functional integration of tissue-engineered bone constructs

R.E. Guldberg[1], M. Oest[1], A.S.P. Lin[1], H. Ito[2], X. Chao[2], K. Gromov[2], J.J. Goater[2], M. Koefoed[2], E.M. Schwarz[2], R.J. O'Keefe[2], X. Zhang[2]

[1]Institute for Bioengineering and Bioscience, School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA, USA,
[2]The Center for Musculoskeletal Research, Department of Orthopaedics, University of Rochester Medical Center, Rochester, NY, USA

Keywords: Tissue Engineering, Biomechanics, Imaging, Bone

Tissue engineering has immense potential to provide significantly improved therapies to patients for the functional restoration of damaged or degenerated bone[1]. The overall goal of these efforts is to develop bone graft substitutes that combine the availability and structural integrity of allograft bone with the osteogenic properties of autograft bone. The strategies being developed are numerous and diverse, reflecting the broad range of potential clinical applications, including, for example, fracture healing, repair of large bone defects, spine fusion, implant fixation, and osteoporosis.

Tissue-engineered bone constructs typically start with a porous biomaterial scaffold that provides a template for repair and may serve as a delivery vehicle for bioactive factors such as cells or growth factors. Bone repair scaffolds may be composed of natural or synthetic materials and must have adequate mechanical properties to withstand site-dependent levels of load-bearing following implantation *in vivo*[2]. Cells capable of osteogenesis may be included to enhance the limited endogenous supply of osteoprogenitor cells[3]. Osteoinductive factors, such as bone morphogenetic proteins or genes, may also be combined directly with scaffolds or delivered via genetically engineered cells. Clearly, interactions among tissue-engineered construct design parameters and the local physiologic environment *in vivo* are critical to whether or not functional integration is successfully achieved. As tissue-engineered constructs become more sophisticated, critical design trade-offs must be made in the selection and integration of cell and scaffold parameters. The scaffold material and architecture that provide optimal mechanical properties, for example, may not be ideally suited for delivering cells and proteins or facilitating the invasion of host cells and vascularity from the surrounding tissue bed *in vivo*.

Clinically relevant *in vivo* models are needed to address these challenging construct design decisions. Although preliminary *in vitro* studies are appropriate for demonstrating initial proof of concept, bone regenerative technologies must ultimately be tested *in vivo*. Bone defect models should be critically-sized, such that they will not heal if left empty[4]. For example, a stabilized 5 mm femoral defect in the rat will heal in some cases when left empty, whereas a 7.5 mm femoral defect will consistently fail to bridge without treatment. Particularly in small animals, *in vivo* test beds should be made as challenging as possible to allow discrimination of the effects of different construct designs. The rat calvarial defect model, for example, can be made critically-sized and is commonly used to test bone repair materials. However, it can not be considered a challenging or discriminatory test bed model since most porous scaffolds easily facilitate complete repair of the defect.

In addition to discriminatory *in vivo* models, quantitative outcome metrics of functional integration are required to fully evaluate bone regenerative technologies. Whereas the tissue engineering field once relied primarily on qualitative observations of tissue type from histological sections, it is now recognized that more quantitative methods of assessing functional integration are needed. With regards to bone repair, such measures may include bone formation throughout the defect, vascular ingrowth, scaffold resorption and bone remodeling, and restoration of mechanical properties.

Three-dimensional non-destructive imaging techniques, such as microcomputed tomography (micro-CT), are increasingly providing a powerful set of tools for quantitative evaluation of new approaches to engineering tissues. For example, micro-CT imaging can be used to quantify parameters of porous scaffold microarchitecture and relate these to mechanical properties or measures of tissue ingrowth[5]. The volume and morphology of bone formation within scaffolds may be quantified either postmortem or monitored over time using recently developed *in vivo* micro-CT systems[6]. Vascular ingrowth during bone repair may also be assessed using an appropriate contrast agent[7]. Importantly, micro-CT analysis is non-destructive, leaving the samples available for additional tests or assays. Quantitative imag-

The authors have no conflict of interest.

Corresponding author: Robert E. Guldberg, Institute for Bioengineering and Bioscience, School of Mechanical Engineering, Georgia Institute of Technology, 315 Ferst Drive, Atlanta, Georgia 30332-0405
E-mail: robert.guldberg@me.gatech.edu

Accepted 4 August 2004

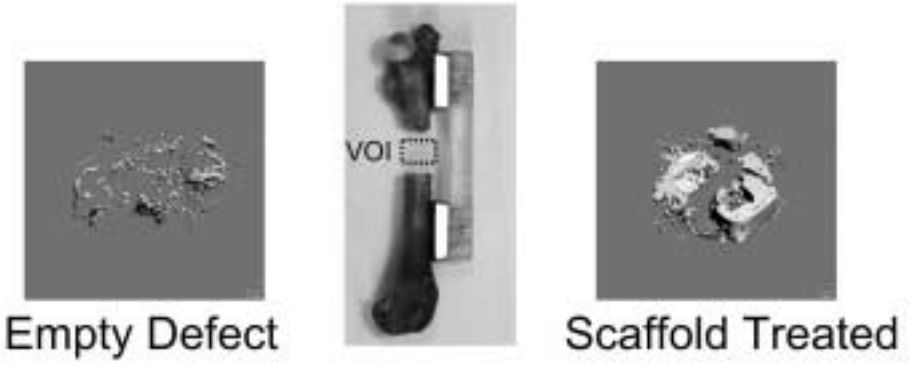

Figure 1. Rat segmental defect model including a polymeric fixation plate that allows longitudinal evaluation of bone ingrowth within a specified volume of interest (VOI) using micro-CT imaging. Micro-CT images of contrast agent perfused animals are shown demonstrating vascular ingrowth only in the empty defect and bone formation in the scaffold treated defect.

ing techniques such as micro-CT can therefore complement histological analysis and mechanical testing to rigorously evaluate, benchmark, and optimize bone tissue engineering technologies.

We have been interested in developing tissue engineering based strategies for repair of large segmental bone defects. Clinically, long bone defects may result from severe trauma or tumor resection. We have recently developed porous poly(L-lactide-*co*-DL-lactide 70:30) (PLDL) scaffolds with a unique transversely isotropic oriented porosity and tested their ability to enhance repair of 5 mm segmental defects in the rat (Lin et al, 2003). Defects were created bilaterally in four rats and either filled with a PLDL scaffold or left empty. After 16 weeks, micro-CT imaging was used to quantify bone and vascular ingrowth into the central 3 mm of the defect region (Figure 1). In some cases, bone formation was observed in the control empty defects, indicating that 5 mm is not a critically-sized long bone defect in the rat. However, scaffold treated defects contained 30% more bone than empty defects. Vascular ingrowth was found to be 17% higher in the scaffold treated defect. Although the results of this study are preliminary, they demonstrate the potential to use micro-CT analysis for quantification of bone and vascular ingrowth into tissue-engineered scaffolds as a measure of functional integration *in vivo*.

We have also used micro-CT analysis to evaluate tissue-engineering strategies for the enhancement of allograft repair of segmental defects. Large structural bone allografts lack significant osteogenic and angiogenic potential, typically leading to incomplete repair and remodeling of bone defects. We tested the hypothesis that wrapping allografts with a cellularized membrane or scaffold prior to implantation would improve the healing response. Bone marrow stromal cells were infected with adenovirus BMP-2, seeded on gelatin scaffolds, and wrapped around devitalized allografts. The revitalized allografts stimulated significantly more new bone formation and vascular ingrowth than allografts alone. Segmentation of newly-formed bone from the implanted allografts revealed an over three-fold increase in bone formation associated with the revitalized allografts. Furthermore, subsequent histological analysis indicated that, unlike the control allografts, revitalized allografts were undergoing substantial bone remodeling, similar to autografts.

In conclusion, micro-CT imaging analysis can be combined with standard mechanical testing and histologic staining techniques to provide a rigorous approach to quantifying functional integration of tissue-engineered bone constructs. Using this approach, pre-clinical studies suggest that tissue engineering based strategies using synthetic or natural scaffolds in combination with bioactive factors may be used to improve the repair of large segmental bone defects.

References

1. Boden SD. Bioactive factors for bone tissue engineering. Clin Orthop Rel Res 1999; 367S:S84-S94.
2. Davy DT. Biomechanical issues in bone transplantation. Orthop Clin North Am1999; 30:553-563.
3. Bruder SP, Fox BS. Tissue engineering of bone. Clin Orthop Rel Res 1999; 367S:S68-S83.
4. Hollinger JO, Kleinschmidt JC. The critical size defect as an experimental model to test bone repair materials. J Craniofacial Surg 1990; 1:60-68.
5. Lin ASP, Barrows TH, Cartmell SH, Guldberg RE. Microarchitectural and mechanical characterization of oriented porous polymer scaffolds. Biomaterials 2003; 24:481-489.
6. Guldberg RE, Ballock RT, Boyan BD, Duvall CL, Lin ASP, Nagaraja S, Oest M, Phillips J, Porter BD, Robertson G, Taylor WR. Microcomputed tomography imaging and analysis of bone, blood vessels, and biomaterials. Eng Med Biol 2003; 22:65-70.
7. Duvall CL, Taylor WR, Guldberg RE. Quantitative microcomputed tomography analysis of vascular growth. Am J Physiol Heart Circ Physiol 2004; [Epub ahead of print].