ESRF	**Experiment title:** In-situ mechanical testing and quantification of the in-vivo mineralisation and vascularisation of bone tissue in macroporous bioactive glass scaffolds	**Experiment number**: MA403
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Report:

The focus of the proposal was to analyse bioactive scaffolds that were implanted into a mouse model. Tomography and holotomography was to be used to compare the structure, tissue ingrowth, mechanical performance and failure models. An existing static compression rig at ESRF was adapted to allow in situ testing in simulated body fluid.

Imaging of immersed and wet samples proved to be problematic due to the photo-chemical cracking of water, produced bubbles which shifted the samples during scanning as a worse case, and in the best case significantly changed the background.

Imaging of dry samples was very successful with the resolution providing excellent contrast such that hard and soft tissue, as well as the scaffold material, could all be differentiated. In addition calcium distribution was detectable within the porous glass scaffold and lacunae could be detected within the mouse bone. This imaging was combined with compression testing very successfully.

Although holotomography was carried out, the analysis of these scans was not successful due to problems in the reconstruction algorithms.

In summary, many portions of the proposal were highly successful, but challenges were met with regards to holotomography and wet imaging. Due to these limitations, a portion of the final day was used on samples ranging from titanium scaffolds to imaging of intermetallics in aluminium alloys. These scans were also highly successful and novel. To date, the work has yielded 7 publications, several of these proving to be very important, as listed below.

1. Jones, J. R., Atwood R. C., Poologasundarampillai, G., Yue, S., Lee, P. D., "Quantifying the 3D macrostructure of tissue scaffolds", J. Mat. Sci.: Mat. in Med 20 (2), pp 463-471, 2009. DOI 10.1007/s10856-008-3597-9

Abstract

The need to shift from tissue replacement to tissue regeneration has led to the development of tissue engineering and in situ tissue regeneration. Both of these strategies often employ the use of scaffolds--templates that allow cells to attach and then guide the new tissue growth. There are many design criteria for an ideal scaffold. These criteria vary depending on the tissue type and location in the body. In any application of a scaffold it is vital to be able to characterise the scaffold before it goes into in vitro testing. In vitro testing allows the cell response to be investigated before its in vivo performance is assessed. A full characterisation of events in vitro and in vivo, in three dimensions (3D), is necessary if a scaffold's performance and effectiveness is to be fully quantified. This paper focuses on porous scaffolds for bone regeneration, suggests appropriate design criteria for a bone regenerating scaffold and then reviews techniques for obtaining the vitally important quantification of its pore structure. The techniques discussed will include newly developed methods of quantifying X-ray microtomography (microCT) images in 3D and for predicting the scaffolds mechanical properties and the likely paths of fluid flow (and hence potential cell migration). The complications in investigating scaffold performance in vitro are then discussed. Finally, the use of microCT for imaging scaffolds for in vivo tests is reviewed.

2. Sheng Yue, Peter D. Lee, Gowsihan Poologasundarampillai, Zhengzhong Yao, Peter Rockett, Andrea H. Devlin, Christopher A. Mitchell, Moritz A. Konerding and Julian R. Jones "Synchrotron X-ray microtomography for assessment of bone tissue scaffolds", J. Mat. Sci.: Mat. in Med 21 (3), pp 847-853, 2010. DOI 10.1007/s10856-009-3888-9

Abstract

X-ray microtomography (μCT) is a popular tool for imaging scaffolds designed for tissue engineering applications. The ability of synchrotron μCT to monitor tissue response and changes in a bioactive glass scaffold ex vivo were assessed. It was possible to observe the morphology of the bone; soft tissue ingrowth and the calcium distribution within the scaffold. A second aim was to use two newly developed compression rigs, one designed for use inside a laboratory based μCT machine for continual monitoring of the pore structure and crack formation and another designed for use in the synchrotron facility. Both rigs allowed imaging of the failure mechanism while obtaining stress–strain data. Failure mechanisms of the bioactive glass scaffolds were found not to follow classical predictions for the failure of brittle foams. Compression strengths were found to be 4.5–6 MPa while maintaining an interconnected pore network suitable for tissue engineering applications.

3. Singh, R., Lee, P.D., Lindley, T.C., Dashwood, R.J., Ferrie, E., Imwinkelried, T., "Characterisation of the structure and permeability of titanium foams for spinal fusion devices", Acta Bio., 5 (1) 2009, 477-487. DOI 10.1016/j.actbio.2008.06.014

Abstract

Titanium foams produced via the space-holder method are used for spinal fusion devices since their combination of an open-cell structure and bone-like mechanical properties promises potentially excellent bone ingrowth. Earlier studies have indicated that the size of the pores and interconnects must be greater than 100 μm for effective bone ingrowth and vascularization. Hence, the quantification of the pore and interconnect size is required for efficient scaffold design. In this study, microcomputed tomography (μCT) was used to obtain the three-dimensional (3D) structure of Ti foams with three levels of porosity (51%, 65% and 78%). Novel algorithms were then applied to quantify both the pore and interconnect size of Ti foams as a function of porosity. All foams possessed a modal pore and interconnect size in excess of 300 μm, satisfying the requirement of being greater than 100 μm. The pore and interconnect size also dominates the flow properties or permeability of open-cell structures. Therefore, the μCT data was also used to generate a mesh for computational fluid dynamics analysis to predict the permeability. The calculated permeability ($117–163 \times 10^{-12}$ m^2 depending on direction) for the Ti foams with 65% porosity was first validated against

experimental measurements (98–163 × 10^{-12} m^2) and then compared to prior authors' measurements in healthy cancellous bovine bone (233–465 × 10^{-12} m^2). The close match among all the permeability values proves the suitability of the material for biomedical skeletal-implant applications.

4. Phillion, A.B., Cockcroft, S.L.; Lee, P.D., "Quantitative assessment of deformation-induced damage in a semi-solid aluminum alloy via x-ray micro tomography", Met. Trans., A 39A(10) (2008) pp2459-2469. DOI 10.1007/s11661-008-9584-4

Abstract

Semisolid tensile testing combined with X-ray microtomography (XMT) was used to characterize the development of internal damage as a function of strain in an aluminum-magnesium alloy, AA5182. Novel techniques were developed to allow the quantification of both the size evolution and orientation of the damage to determine mechanisms controlling the early stage growth and localization. During the initial stages of semisolid deformation, it was observed that strain was accommodated by both the growth of as-cast porosity and the detection of new damage-based voids. As the volume fraction of damage increases, the growth of voids occurs in an orientation perpendicular to the loading direction, both through expansion within the grain boundary liquid and void coalescence. The damage then localizes, causing failure.

5. Atwood, R.C., Lee, P.D., Konerding, M.A., Rockett, P. and Mitchell, C.A., "Quantitation of Microcomputed Tomography-Imaged Ocular Microvasculature", Microcirculation, 2010 (17), 59–68. DOI 10.1111/j.1549-8719.2009.00009.x

Abstract

Purpose: To quantitatively assess microvascular dimensions in the eyes of neonatal wild-type and $VEGF_{120}$-tg mice, using a novel combination of techniques which permit three-dimensional (3D) image reconstruction.

Methods: A novel combination of techniques was developed for the accurate 3D imaging of the microvasculature and demonstrated on the hyaloid vasculature of the neonatal mouse eye. Vascular corrosion casting is used to create a stable replica of the vascular network and X-ray microcomputed tomography (μCT) to obtain the 3D images. In-house computer-aided image analysis techniques were then used to perform a quantitative morphological analysis of the images.

Results: With the use of these methods, differences in the numbers of vessel segments, their diameter, and volume of vessels in the vitreous compartment were quantitated in wild-type neonatal mice or littermates over-expressing a labile (nonheparin binding) isoform of vascular endothelial growth factor ($VEGF_{120}$) from the developing lens. This methodology was instructive in demonstrating that hyaloid vascular networks in $VEGFA_{120}$ over-expressing mice have a 10-fold increase in blind-ended, a six-fold increase in connected vessel segments, in addition to a sixfold increase (0.0314 versus 0.0051 mm^3) in total vitreous vessel volume compared with wild type. These parameters are not readily quantified via histological, ultrastructural, or stereological analysis.

Conclusion: The combination of techniques described here provides the first 3D quantitative characterization of vasculature in an organ system; i.e., the neonatal murine intra-ocular vasculature in both wild-type mice and a transgenic model of lens-specific over-expression of VEGF.

6. Lee, PD, Wang, J, Li, M, "Coupling In-situ Observations and Microscale Modeling to Predict Pore and Fe-rich Intermetallic Formation during the Solidification of Al-Si-Cu-Fe Alloys", Modeling of Casting, Welding and Advanced Solidification Processes - XII, Vancouver, June 2009, p87. (C1). DOI

The presence of pores and intermetallics can often limit the fatigue performance of cast aluminum alloy components. However, predicting the size distribution of these microstructural features is difficult due to the stochastic nature of their nucleation, complex thermodynamics/kinetics, and their interactions with other phases during growth. This paper will investigate how *in situ* X-ray observations (using synchrotron and lab sources) can be combined with three dimensional microstructural models to predict the size, morphology and

distribution of the Fe-rich intermetallic phases (e.g. α-Al_8Fe_2Si and β-Al_5FeSi) and pores which form during the solidification of Al-Si-Cu-Fe alloys.

The kinetics of Fe-rich intermetallic formation is unknown due to the highly non-equilibrium nature of this process which makes it difficult to determine the local undercooling using normal quenching techniques. Because of their fine faceted shapes, direct measurements of their kinetics are only possible when high resolution imaging (e.g. 1 μm/pixels) is adopted. In-situ observation using synchrotron X-ray radiography allows both Fe-rich intermetallic and pore nucleation and growth kinetics to be quantified as a function of temperature. Using this experimental data, we developed a kinetic-thermodynamic model, solving both multicomponent diffusion equations and total free energy. Crystallographic anisotropies of monoclinic β-Al_5FeSi are accounted for by an adapted implementation of combined Monte-Carlo and geometric constraint techniques. The resulting simulations predict that increasing the Fe content causes the Fe-rich intermetallics to form large needles/plates, in agreement with synchrotron tomography results. Increasing Fe content has a non-linear effect on pore formation, first increasing and then decreasing the pore size. This non-linear effect is hypothesized to be caused by the interaction of competing influences on pore nucleation and growth rates.

7. Junsheng Wang, Peter D. Lee, "Quantitative Simulation of Fe-rich Intermetallics in Al-Si-Cu-Fe Alloys during Solidification", Frontiers in Solidification Science III Edited by Ralph E. Napolitano, & James R. Morris, TMS (The Minerals, Metals & Materials Society), 2009.

Fe-rich intermetallic phases (e.g. β-Al_5FeSi), which form during the solidification of Al-Si-Cu-Fe alloys, can have a detrimental effect on the mechanical properties of cast components. A thermodynamic database was combined with a numerical solution of multi-component diffusion to predict the formation of both this secondary phase and the primary phases. The growth kinetics and morphology was simulated using a combined Monte-Carlo algorithm to calculate the transformation frequency for each cell as a function of total free energy, with the faceted shapes replicated *via* a decentred needle/plate algorithm. The non-stoichiometric composition of both Fe and Si was simulated by changing the partition coefficient at the solid/liquid interface according to local kinetic parameters such as growth velocity and effective diffusion. The model predicted phase fraction, size and morphology of Fe-rich intermetallic phases in type-319 alloys (Al-7.5wt.%Si-3.5wt.%Cu-0.2~0.8wt.%Fe) compared well to high resolution synchrotron tomography observations.