MICRO-CT DETERMINATION OF BONE INGROWTH INTO POROUS BIOCERAMICS

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ABSTRACT

For tissue engineering of bone to be successful, it is essential that the growth of bone into scaffolds be monitored accurately and in three dimensions. Microcomputed tomography is a suitable tool for this application; however, the technology for processing and analysing the images and data are not well developed. This paper examines the use of advanced image processing technology to investigate bone growth into porous bioceramic implants from a sheep model after 4 weeks. Challenges that are overcome include: accurate phase separation despite x-ray adsorption of the ingrowing tissue closely matching that of the scaffold, specifying tissue mineralisation profiles and the development of metrics that can describe in situ scaffold pore analysis and local bone healing parameters.

KEY WORDS

Micro-computed tomography, image analysis, bone ingrowth, alumina, hydroxyapatite, sheep

1. Introduction

Bone regeneration by tissue transplantation is regarded as one of the most promising techniques in orthopedic surgery and biomedical engineering. Treatments based on engineered tissues will alleviate the vast shortage of donor tissue and would eliminate problems of donor site cross-infection, immune rejection and scarcity[1-3]. Almost all tissue engineering concepts require the use of some form of porous scaffold [eg. 4-8 ], which serves as a template for cell proliferation and, ultimately, tissue formation. Porous bioceramics, especially those based on calcium phosphates, form a class of potential scaffolds that can provide early load bearing, well before full integration of the tissue engineered construct into the patient’s repair site.

Control of pore size and morphology, mechanical properties, flow and diffusion properties is critical for controlling mechanical stability, cellular colonization rates and organization within an engineered scaffold/tissue construct.

Important questions remain however;
- What scaffold designs perform best and why?
- Can we design an optimal scaffold architecture?
- When does the grown scaffold/tissue construct obtain similar mechanical properties to the host bone?

These questions are crucial to clinical and industrial researchers developing engineered tissue. Answering these question requires the ability to image the microstructure and to measure material properties of the complex composite during the full implantation period. Conventional testing of in-vitro and in-vivo tissue engineered scaffold cell constructs is based on destructive 2D structural measurements (e.g., freeze fracture microscopy) and irreversible mechanical testing. Information about 3D distributions of bone ingrowth or properties are rarely gathered from such 2D investigations, simply due to the enormity of the task. MicroCT already has demonstrated the potential to image tissue ingrowth and calcification into porous polymeric scaffolds [9, 10] in 3D. For small samples, the technique can also have the resolution to visualize small canals (blood vessels)[11]. Visualisation of calcifying tissue in biocermics, where the electron density is more similar, is a more difficult task. This paper reports the development of techniques to enable measurement of bone ingrowth into porous bioceramics and also to directly measure the scaffold in situ.

2. Materials and Methods

2.1 Materials

All chemicals used were of reagent grade. For porous hydroxyapatite (nominally 60% and 70% porous by volume fraction): Hydroxyapatite (Hap) was sourced from Merck. Naphthalene was crushed and sieved to give particle sizes of approximately 300 micrometres. The calcined HAp (900 °C for 1 hour) was ground to give particle sizes around 1 to 10 micrometres. Two types of porous hydroxyapatite (HAp) were prepared by mixing calcined and ground HAp with sieved naphthalene to give volume fractions of 0.30 and 0.40 HAp respectively. The
mixtures were pressed in a 25 mm diameter cylindrical mould to a total force of 15 tonnes. The resulting greens were then heated to 300 °C for 1 hour to remove the naphthalene prior to firing at 1200 °C for 3 hours.

Porous alumina samples were prepared mixing polyethylene glycol (PEG) particles (Sigma Chemical CO, MW 8000, 200 to 400 micrometre diameter) in a 0.45:1 w/w ratio. Bars of the mixture, final size 20 mm by 50 mm by 5 mm, were pressed in moulds at 25kN then cold isostatically pressed at 50 MPa. The PEG was burned out at 800 °C for 2 hours prior to final firing at 1600 °C for 2 hours.

2.2 Animal model.
All procedures were approved prior to commencement by the UNSW Animal Care and Ethic Committee and followed NH&MRC Guidelines on the Use of Animals. Two skeletally mature wethers of 40 to 60 kg were used. Bilateral operation was allowed as the procedure has been proven complication free in healthy animals. The sheep were prepared for the operation in the manner outlined below. After pre-operative preparation and induction of anaesthesia, each sheep was placed in dorsal recumbancy and the medial surface of each tibia was exposed.

Seven holes, 3 mm in diameter, are drilled into the medial surface 2 cm apart using an air-powered drill and 3 mm bone drill bit with continuous saline irrigation. Holes were then reamed square with a square section broach or file. Each hole was filled with a sample piece as randomly assigned previously. Sample pieces were a push fit and left flush or slightly proud (no more than 1 mm) with the outer cortical bone surface. The periosteum was sutured over the pieces and the skin sutured closed. The animals were euthanased at 4 weeks post-operatively and the samples collected with surrounding bone into 4% buffered formalin. Some samples were used for mechanical testing. One sample of each type was kept for microCT evaluation.

2.3 Micro Computed tomography
The microCT device was developed and built at the Department of Applied Mathematics, Australian National University [12] with a cone beam geometry, which allows adjustable magnification. The scaffold constructs were imaged wet at an X-ray accelerating voltage of 80 kV, with a 1.24 mm thick aluminium filter placed over the source to reduce beam hardening artifacts. A total of 1800 projections were captured over 360 degrees and were binned to 1024 x 1024 16-bit gray level images. The projection data were reconstructed by locally written software using standard algorithms (Feldkamp technique [13]) to yield 1024³ voxel tomograms with the attenuation coefficient at each voxel (tomographic volume element) normalised over the interval [0,2^{16}). Although the chemical composition of bone and the hydroxyapatite scaffolds are similar, the physical density of the scaffolds varied significantly from the bone and may enable phase separation based on attenuation coefficients.

By using the X-ray attenuation as an indicator of the mineralization, we trace contours of the degree of mineralization within the tissue in the scaffold.

3. Results and Discussion
Figure 1 shows the normalised attenuation coefficient histograms for each of the scaffold constructs. The two right most peaks correspond to the scaffold and mineralised tissue phases respectively. The contrast between the scaffold and mineralised tissue varies with the specimen: in the nominally 70% porous HAp specimen there is much greater contrast than in the porous alumina specimen.

![Figure 1](image1.png)

3. Results and Discussion
Figure 1: Histogram of attenuation coefficient values for 70% porous HAp (solid line), 60% porous HAp (dashed line), and porous alumina (dotted line). The two right most peaks typically correspond to scaffold and bone.

![Figure 2](image2.png)

3. Results and Discussion
Figure 2: (left to right) 70% Porous HAp, 60% Porous HAp, Porous alumina. The periosteal side is shown on the left in all images.

Also in Fig 2, the representative slices from the reconstructed tomograms show density gradients of the mineralised tissue across the specimens. The inhomogeneity of mineralised tissue densities is a difficulty for any subsequent thresholding and image processing. A contour map can present a qualitative understanding of the mineral density gradients within the porosity of the scaffold. Fig. 3 shows a close up of the 70% porous HAp scaffold with a mineralised tissue contour map of the same region.

Image phase separation of scaffold material from the mineralising bone can be problematic. For the
Hydroxyapatite (HAp) scaffolds the attenuation peak associated with HAp is distinct allowing one to differentiate much of the scaffold from the bone. However, voxels at the HAp/Air interface exhibit intermediate attenuations and phase separation based on simple thresholding can lead to phantom rings of mineralised bone (see Fig. 4) lining the HAp/air interfaces. We utilise a novel multistep image enhancement and phase separation technique to remove the phantom and to give a realistic image of the HAP, bone and air distribution in 3D (Fig.4). Clearly the phase separation into the three phases shown in the right hand image of Fig. 4 is excellent.

![Figure 3](image1.png)

**Figure 3.** Left: Cross section through 70% porous HAp scaffold showing attenuation coefficient gray level. Right: attenuation coefficient contour map around HAp implant illustrated for same cross section. A range of densities is apparent for the mineralising tissue ingrowth.

![Figure 4](image2.png)

**Fig. 4: Left:** Original grey scale image of one slice of the 70% porosity HAp scaffold with bone ingrowth. Middle: Phase separation of the slice based on more primitive phase separation techniques. Bone phase is shown in black. Note the presence of “bone” phase at the border between HAp and air. Right: Three phases based on more advanced phase separation leads to a more realistic distribution of HAp (grey), air (white) and bone (dark).

The scaffolds may be analysed for pore geometry, size and other metrics such as throat size (an important factor for bone ingrowth). This allows the bone ingrowth data to be compared with the actual scaffold geometry, rather than inferred geometry from exemplar pieces. We first consider the fraction of mineralised bone within the scaffold along three orthogonal axes (Fig. 5). Along the z-axis (periostial to endostial axis) we observe higher bone content in regions of higher porosity (lower HAp density).

![Figure 5](image3.png)

**Fig. 5: Top left:** Fiducial region—region over which the density is measured. Top right: Bone and HAp density along the Periosteal to endostial bone axis. Densities in orthogonal directions are shown on the lower figures.

We also consider a model free technique for defining the pore and solid size distributions of a 3D image which is based on defining locally, for every point within the structure, the diameter of the largest sphere which fully lies within the pore and solid phase and covers that point [11,14]. Example of the embedded sphere map for a slice of the original scaffold are given in Fig 6. Data for the pore space before bone ingrowth, bone growth and pore space after growth can be reported using this technique. In addition, other metrics may be derived from the bone density contour data, including variability within the scaffold in relation to the anatomical position.

![Figure 6](image4.png)

**Fig. 6: Illustration of the measurement of pore/solid size distribution of a 3D image using a covering sphere map.** In both images the field of covering spheres for each phase is given with grey scale proportional to sphere radius (white is largest sphere, dark is smaller sphere). Left shows the covering sphere map for a slice of a solid phase, and right shows the map for the pore phase.
4. Conclusion

We have demonstrated the potential of microCT to distinguish HAp scaffolds from mineralised ingrowth using differences in physical density. This is not the case, however, for porous alumina, which had insufficient difference in density from the surrounding bone tissue. Techniques are developed to overcome this problem. Scaffold morphometric parameters (eg. pore size, interconnectivity/throat size) can then be determined from the segmented scaffolds regions. The mineralising tissue gradients within the scaffolds can also be quantitatively described using contour mapping and slice-by-slice volume fraction measurements (Figures 3 and 5) and may be used as the basis for quantitative studies of mechanical properties and bone growth analysis.

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