Non-destructive quantification of tissue scaffolds and augmentation implants using X-ray microtomography

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DECLARATION

I hereby certify that the work presented in this thesis is the result of my own investigations carried out at Imperial College London during the period from January 2008 to September 2010, except where otherwise stated.

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ABSTRACT

A three dimensional (3D), interconnected, porous structure is essential for bone tissue engineering scaffolds and skeletal augmentation implants. Current methods of characterising these structures, however, are limited to average properties such as percentage porosity. More accurate quantitative properties, such as pore and interconnect size distributions, are required. Once measured, these parameters need to be correlated to tissue regeneration and integration criteria, including solute transport, blood vessel regeneration, bone ingrowth, and mechanical properties. Ideally, these techniques would work in vitro and in vivo, and hence allow evaluation of osteoconduction and osseointegration after implantation.

This thesis will focus on developing and applying algorithms for use with X-ray microtomography (micro-CT or µCT) which can non-destructively image internal structure at the micron scale. The technique will be demonstrated on two separate materials: bioactive glass scaffolds and titanium (Ti) augmentation devices.

Using the developed techniques, the structural and compositional evolutions of bioactive glass scaffolds in a simulated body fluid (SBF) flow environment were quantified using micro-CT scans taken at different dissolution stages. Results show that 70S30C bioactive scaffolds retain favourable 3D structures during a 28 d dissolution experiment, with a modal equivalent pore diameter of 682 µm staying unchanged, and a modal equivalent interconnect diameter decreasing from 252 µm to 209 µm.

The techniques were then applied to porous Ti augmentation scaffolds. These scaffolds, produced by selective laser melting have very different pore networks with graded randomness and unit size. They present new challenges when applying the developed micro-CT quantification techniques. Using a further adapted methodology, the interconnecting pore sizes, strut thickness, and surface roughness were measured. This demonstrated the robustness of the methodologies and their applicability to a range of tissue scaffolds and augmentation devices.
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PUBLICATIONS ARISING FROM THIS THESIS

During the course of my PhD studies, several portions of this thesis have already been submitted and published in journal articles and presented at conferences, as follows.

Journal publications


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Oral presentations


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1. **INTRODUCTION**

Musculoskeletal tissue defects, caused by disease or injury, are one of the most devastating and expensive problems in health care. The current strategies to deal with these problems include: autologous or allogeneic transplantation, grafting with synthetic materials, and tissue replacement by mechanical devices.

Although millions of patients benefit from these therapeutic options each year, many limitations and unsolved problems remain (Shieh and Vacanti, 2005). For example, the current gold standard to repair bone loss is an autograft. In autologous bone grafting, cancellous bone (also called trabecular bone in certain sites), normally harvested from the iliac crest of the same patient, is used to repair large bone loss. These autografts not only act as scaffolds, but also provide osteogenic factors such as bone morphogenetic proteins (BMPs) and the transforming growth factor beta (TGFβ) (Revell, 2008). The main restriction of autografting is limited material availability from donor site(s). Also, the donor site must be repaired and side effects can occur such as chronic pain at the donor site, which may result in a revision operation for the donor site (Revell, 2008).

Alternatives include allogeneic and artificial grafts. Allogeneic grafts are usually irradiated bone obtained from either cadavers (Nather, 2005). However, their mechanical properties are poor and there is a risk of disease transmission and rejection. Many types of artificial bone grafts and bone graft extension materials have also been developed. For bone defects under high load a metallic bone analogue may be needed to augment or replace the bone. However for other defects the aim is to mimic bone and regenerate it to its natural state and function using bone regeneration, using an artificial bone graft, or tissue engineering strategies. In bone tissue engineering, osteogenic cells harvested from the patient are expanded in culture and placed onto a synthetic scaffold, which acts as a support and a guide for *in vitro* tissue regeneration. The tissue-scaffold construct is then implanted at a later state to the defect site once enough tissue has been generated (Hench and Polak, 2002).
The most popular and successful synthetic graft materials are macroporous bioactive ceramic granules, such as calcium sulphate, tricalcium phosphate (β-TCP), synthetic hydroxyapatite (HA), and biphasic calcium phosphate (a mixture of TCP and HA). The problem with these materials is the dissolution rate: they either dissolve rapidly (calcium sulphate and TCP) after implantation, which causes clinical problems, or degrade very slowly (HA) (Jones et al., 2010).

The design of these hard tissue scaffolds and augmentation devices has focused on porous structures in general, ranging from metallic to ceramic and polymer. An interconnected porous architecture can enable solute transport, blood vessel penetration, and bone ingrowth after implantation, providing integration with the local host site via biological fixation. In order to achieve that, these porous structures need to fulfil many criteria. For example, the cellular response of the scaffold in vitro and in vivo has been shown to correlate to structural parameters such as pore and interconnect size distributions, surface area to volume ratio, and surface topology of the scaffolds. Therefore, accurate characterisation of the porous architecture is important to evaluate and predict the performance of bone scaffolds and help to optimise their structure design.

The objective of this thesis was to develop image processing algorithms for the quantification of different types of interconnected pore networks. The primary source of scaffold images used in this study has been obtained using X-ray microtomography (micro-CT), which can non-destructively image scaffolds in three dimensions (3D).

The structure of this thesis is organised as follows:

The background and prior literature on porous bone scaffold design is provided and reviewed in Chapter 2, with special attention paid to the 3D image based quantification methods.

Chapter 3 describes the core methodologies developed and applied throughout this study, specifically the use of micro-CT coupled with novel image processing algorithms. This is demonstrated first on the quantification of bioactive glass scaffolds. The quantification accuracy is shown to dramatically improve over previous studies by using a principal component analysis based method. The natural orientation of an interconnect in the foam scaffold can be effectively located and therefore the size of the interconnect can be measured accurately and efficiently.
In Chapter 4, this quantification methodology is applied to evaluate the *in vitro* dissolution behaviour of 3D bioactive glass scaffolds. The improved accuracy is shown to be essential for accurately quantifying the degradation of strut material as well as structural variation in 70S30C scaffolds.

In Chapter 5, the quantification method is extended to titanium foam bone augmentation devices. These structures have graded levels of randomness built into their geometry, and required further novel extensions in the methodology for quantification of the pore and interconnect size distribution and the development of algorithms to analyse their surface roughness. The application of these methods is then presented in Chapter 6, together with a discussion of the implications for product quality control and assurance.

The overall conclusions from this thesis are presented in Chapter 7, together with recommendations for future work in Chapter 8.

Finally, the core Matlab code segments used for structure quantification are given in Appendix A.
2. **LITERATURE REVIEW**

2.1. **Tissue engineering strategy**

Bone is a dynamic, highly vascularised tissue (Figure 2.1) that has the ability to heal itself when the defect is small (Kneser et al., 2002). Restoring large bone loss, due to trauma, infection or disease, is a major issue for healthcare (Kneser et al., 2006). Current clinical procedures to repair large bone defects, including transplantation or implantation, are facing problems such as availability, functionality, rejection by the body, and long-term failure (Berry et al., 2002, Jones et al., 2006a).

![Figure 2.1. Illustration of human bone structure. Modified from Human body I, 2009, Encyclopaedia Britannica, Chicago.](image)

For example, the current ‘gold standard’ in reconstructive surgery for damaged or diseased bone is autologous bone grafting. The effectiveness of this method is limited by donor-site morbidity, increasing with the amount of harvested bone as a result of bleeding, haematoma, infection, chronic pain, and unpredictable bone resorption (Kneser et al., 2002, Kneser et al., 2006, Burg et al., 2000). Therefore, synthetic alternatives are needed. For high load bearing
sites, metallic materials must be used, but they may only have a limited lifetime. Ideally, a shift is needed from bone replacement to bone regeneration (Hench and Polak, 2002).

One strategy for restoring diseased or damaged tissue to its original state and function is tissue engineering (TE). The definition of tissue engineering that is widely accepted was given by Langer and Vacanti (Langer and Vacanti, 1993) in 1993 as “an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function.” This concept was loosely applied in 1980s (Vacanti, 2006) to the use of prosthetic devices and the surgical manipulation of tissues and the term tissue engineering was first used in 1991 (Vacanti, 2006).

The strategy of tissue engineering generally involves the following steps (Langer and Vacanti, 1993, Langer, 2000):

i. Appropriate cell sources must be identified, isolated, and produced in sufficient amount.

ii. Appropriate biocompatible materials that can be used as a cell substrate (open system) or cell-encapsulation material (closed system) must be manufactured into the desired shape and dimensions.

iii. The cells must be uniformly seeded onto or into the material and grown in a bioreactor.

iv. The engineered structure is placed into the appropriate in vivo site. Depending on the site and the structure, vascularisation may be necessary.

When applying this scheme to repair large bone defect, an ideal scenario (Ohgushi and Caplan, 1999, Jones et al., 2006b) could be harvesting stem cells or osteoprogenitor cells from the patient. The autologous cells are then expanded in culture and seeded on a template scaffold in a bioreactor system. Once enough new tissue has been generated in vitro, the tissue/scaffold composite can then be implanted to the defect site of the patient. The biocomposite is then expected to bond to the local tissue in vivo, and encourage bone ingrowth and vascularisation so that it can be gradually replaced by the patient’s own bone.
2.2. Design criteria of bone scaffolds

In general, a bone tissue engineering scaffold should act as a template for cell attachment, expansion, and subsequent tissue regeneration during in vitro culture. When implanted in vivo, it also needs to support load, bond to the host site, and encourage bone ingrowth and vascularisation (Hutmacher, 2000, Jones and Hench, 2003b, Hollister, 2005, Jones et al., 2006b). To achieve these goals, the design of the scaffold must fulfil several main criteria as following.

i. A bone scaffold should possess a porous, interconnected structure with preferred surface topology (Jones et al., 2006b, Ho and Hutmacher, 2006, Jones et al., 2007c, Jones et al., 2009a).

ii. To avoid stress shielding, the mechanical properties of the implanted scaffold/tissue biocomposite should match those of the host site.

iii. The material used to fabricate bone scaffold needs to be biocompatible. That is, the material and its in vivo degradation products should be non-toxic to the body, both chemically and physically.

iv. The biomaterial should also promote cell adhesion and activity, stimulating bone regeneration both in vitro and in vivo. Once implanted, it should quickly bond to the host site to achieve a stable fixation.

v. Ideally the implanted scaffold will degrade at the same rate as new tissue is generated in vivo. And the degradation products can encourage bone regeneration (Jones et al., 2006b, Jones et al., 2007c).

vi. The scaffold needs to be commercially produced and sterilised, so that it can meet international regulations for clinical use (Jones et al., 2006b).

Various materials have been used to fabricate bone scaffolds, including natural materials, polymers, ceramics, metals, or combination of these materials. The influences of their chemical compositions on cellular response and local site pathology have been intensively studied. In this section, attention will be mainly drawn on how structural parameters can affect the performance of the scaffold.

Porosity is defined as the percentage of void space in a solid, which is an important parameter for scaffold design as it directly affects the transport property of the scaffolds (Karageorgiou
and Kaplan, 2005). Successful exchange of oxygen, nutrient, metabolic wastes and molecular signalling is essential for cell migration and proliferation into the scaffold (Botchwey et al., 2003). The solute exchange in the tissue/scaffold biocomposite in vitro and in vivo is mainly achieved by perfusion and diffusive flow (Ho and Hutmacher, 2006); and high interconnecting porosity can significantly enhance the permeability of the scaffold. In addition, high porosity and high surface area to volume ratio are required for uniform cell delivery, cellular attachment and neo-tissue ingrowth (Freed et al., 1994, Mooney et al., 1996, Gauthier et al., 1998, Kim and Mooney, 1998).

Porosity also has negative influence on the mechanical strength of bone scaffolds. This means increasing porosity can be used to reduce stiffness in metals, such as Ti and its alloys. However, ceramic or glass scaffolds with high porosity may have poor mechanical properties. For example, the mechanical strength of highly porous (porosity > 90 %) GC-Bioglass scaffolds made by polymeric sponge replication method (Chen et al., 2006) was an order of magnitude lower than that of cancellous bone.

A key feature for a hard tissue scaffold is an interconnected pore network, which allows cell migration, and subsequently cell proliferation as well as formation of extra cellular matrix (Bonfield, 2006). The macro pores inside the scaffold provide space for cell attachment and new tissue regeneration. The interconnects between the pores control the size of available pathway for new tissue and blood vessel penetration. Early study shows that a minimum pore size of 100 μm is required for bone regeneration (Hulbert et al., 1972, Klawitter and Hulbert, 1971). More recent works suggest that the pore size should be 300 – 600 μm (Hutmacher et al., 2004, Jones et al., 2009a, Schwartz et al., 1995, Okii et al., 2001) and the interconnect diameter should be greater than 200 μm (Lu et al., 1999, Flautre et al., 2001, Otsuki et al., 2006). However, exact minimum pore and interconnect values are not possible to obtain without systematic clinical trials.

The geometrical features of the pores, such as surface area and local curvature, can influence the local growth rate of the tissue in a scaffold. High surface to volume ratio can promote cell adhesion. Rumpler et al. compared the tissue formation after 21 and 30 days of cell culture in channels with triangular, square, hexagonal, and round shape in a hydroxyapatite (HA) plate in vitro. Together with mathematical modelling, their results suggested that the local growth rate was proportional to local curvature in the scaffold (Rumpler et al., 2008).
2.3. **Structure quantification methods**

As discussed in section 2.2, structural parameters of bone scaffolds play key roles in affecting bone ingrowth, vascularisation, solute transport, and mechanical strength. Accurately quantifying these structural parameters, can help researchers to understand how the performance of the scaffold being influenced, and therefore can help the optimisation of the scaffold design.

**Optical microscopy and scanning electron microscopy (SEM)**

Optical microscopy and scanning electron microscopy (SEM) are commonly used to directly visualise the morphology of a scaffold (Habibovic et al., 2006, Mather et al., 2008). These techniques provide high resolution, highly detailed surface topology of the sample (Ho and Hutmacher, 2006). With X-ray microanalysis in an SEM (e.g. energy-dispersive X-ray spectroscopy, EDS or EDX), the elemental distribution at the viewing cross section can also be provided.

However, the main drawback of 2D imaging is that the views are limited to the superficial regions. Only images of a fracture surface can be obtained and the imaging regions are relatively small (Ho and Hutmacher, 2006). The porosity and pore size distribution can be estimated from a cross-section; but the interconnect size is difficult to be obtained from 2D images. In addition, these techniques are destructive and the samples normally need to be prepared before imaging.

**Mercury intrusion porosimetry (MIP)**

Mercury intrusion porosimetry (MIP) has been intensively used to evaluate the porous nature of a material (Pilliar et al., 2001, Sepulveda et al., 2002a, Giesche, 2006). Porosity, pore size distribution, and the surface area (internal) of an open, well interconnected scaffold can be obtained from MIP. However, the pore size distribution derived from MIP data actually reflects the interconnections (i.e. interconnects, or pore channel) but not the macro pores in the structure (Giesche, 2006); therefore the measured size is always smaller than similar data obtained from optical microscopy or SEM.
There are several limitations of MIP (Ho and Hutmacher, 2006, Otsuki et al., 2006, Jones et al., 2007c, Giesche, 2006): 1) the technique only works with pore sizes ranging from 2 nm to 400 µm and it cannot take into account isolated (closed) pores; 2) the process is invasive and destructive, and may deform the sample due to the applied pressure; 3) some information obtained, like size distribution and surface area, are indirect and the morphology of the pores remains unknown; and 4) the technique is sensitive to surface roughness of the sample, and Washburn equation (the equation used to related applied pressure and pore size in the sample (Washburn, 1921)) assumes prefect cylindrical pores in the sample which is usually not the case in reality.

*X-ray microtomography (micro-CT)*

High resolution X-ray microtomography (micro-CT, XMT or XRT) is a powerful tool for materials characterisation. It can image the X-ray attenuation variation within an object at a micron-level resolution with a fast, non-invasive, and non-destructive process (Stock, 1999, Stock, 2008). The X-ray attenuation map closely reflects the composition and density of the material. Therefore, with 3D image analysis techniques, it is possible to visualise, and quantify the internal structure of the scaffold.

Like other computed tomography (CT) techniques, a micro-CT image is generated by rotating the sample in very small steps (<1°) around a single axis of rotation while taking a series of 2D radiographs. Afterwards, a 3D image can be numerically reconstructed from these radiographies using reconstruction algorithms, such as filtered backprojection algorithm (Feldkamp et al., 1984), and can be saved either as a series of section images or a volume file.

The physical basis of micro-CT is that material attenuates the X-ray passing through it. The attenuation rate depends on X-ray energy and composition of the object. When a suitable range of energy is used so that the attenuation is mainly contributed by the photoelectric effect, the intensity of the transmitted X-rays is related to the incident X-ray intensity by (Stock, 1999):

\[
I = I_0 e^{-\mu \rho x}
\]

where, \(I_0\) is the intensity of the unattenuated X-ray beam, and \(I\) is the beam’s intensity after it traverses a thickness of material \(x\) characterised by a linear attenuation coefficient \(\mu\). The
term $\mu/\rho$ is the mass attenuation coefficient (cm$^{-2}$ g$^{-1}$) of the object and the equation can be rewritten as:

$$\frac{\mu}{\rho} = -(\rho x)^{-1} \ln \left(\frac{I}{I_0}\right)$$  \hspace{1cm} 2.2

The mass attenuation coefficient is a function of the incident X-ray photon energy, density and composition of the material. The term $\rho x$ is the mass density of the object. An object made up of different constituents, with differing mass densities, will attenuate the incident radiation accordingly to form a radiograph. Figure 2.2 shows mass attenuation coefficients of several common materials related to clinical CT and bone scaffold characterisation.

![Figure 2.2. Mass attenuation coefficients for different materials. Data obtained from http://www.nist.gov/pml/data/xraycoef/index.cfm, (Hubbell and Seltzer, 1995).](image)

Adding the increments of attenuation along the direction of X-ray propagation yields the more general form of Equation 2.1:

$$I = I_0e^{-\int \mu(s)ds}$$  \hspace{1cm} 2.3

where $\mu(s)$ is the linear absorption coefficient at position $s$ along ray $s$. 
Reconstruction is the process to assign the correct values of $\mu$ to each position of the ray, when knowing only the values of the line integral for the various orientations of $s$, i.e.:

$$\int \mu(s) ds = \ln \left( \frac{l_0}{l} \right)$$  \hspace{1cm} 2.4

The most common reconstruction method used in modern CT system is filtered backprojection algorithm (FBP) (Feldkamp et al., 1984), which is based on the Fourier space reconstruction method. The fundamental theory of Fourier space reconstruction is the Fourier slice theorem (also termed as projection-slice theorem, central slice theorem), which is stated as (Hsieh, 2009):

“The Fourier transform of a parallel projection of an object (denoted as $f(x,y)$) obtained at a particular angle equals a line in a 2D Fourier transform of $f(x,y)$ taken at the same angle.”

If a series of parallel projections (i.e. radiographies) over the range from 0 to $\pi$ with an infinite small angle interval has been collected, the entire Fourier space of the object is filled so that the attenuation map of the object can be recovered by an inverse Fourier transform. In practice, only a finite number of projections are collected and the reconstruction process including the re-gridding of the polar gridded ray integral data to Cartesian coordinate in the Fourier domain by interpolation, which is not as straightforward as interpolation in real domain. Therefore, a similar method called filtered backprojection algorithm (Feldkamp et al., 1984) is commonly used for tomographic reconstruction by applying frequency filters before the inverse Fourier transform to tackle this problem and improve the reconstruction accuracy. When a cone beam (Figure 2.3a) X-ray source is used instead of a parallel beam (Figure 2.3b), the projections need to be collected over the range from 0 to $2\pi$.

Micro-CT can be divided into two types with respect to X-ray sources: synchrotron radiation and Micro/Nano focus X-ray tube heads. Monochromatic synchrotron X-ray radiation is generated from an electron storage ring. The released X-rays can have a specific wavelength in a very wide range from $10^{-5}$ m to $10^{-13}$ m. Rather than a point source, a finite size source (parallel) is commonly used in synchrotron micro-CT, which can significantly speed up the scanning process without geometric magnification (Bonse and Busch, 1996). Micro/Nano focus X-ray tube heads generate X-rays by the bombardment of electrons on to a target, and
point source with a projected cone bean is commonly used. The X-rays generated by a Micro/Nano focus X-ray tube head is polychromatic (white X-rays) and is possible to cause beam hardening artefact.

![Figure 2.3](image)

Figure 2.3. Illustration of two primary experimental approaches to micro-CT data collection: (a) cone beam used at lab based facility and (b) parallel beam used at synchrotron source.

The tomographic reconstruction is a mathematical approximation process and the accuracy and quality of the obtained volume can be limited by X-ray source, detector, sample size, as well as number and quality of the radiographies.

The source and energy used can significantly affect the image quality, which is usually assessed by contrast and resolution. Contrast is defined as the ratio of difference in signal between the object and background to signal of background, as shown in Equation 2.5. The higher the contrast, the better the distinction between object phase and background phase in the histogram of the image. Low contrast may be improved by infiltrating contrast agents into specimens (Stock, 2008).

\[
\text{Contrast} = \frac{I_f - I_b}{I_b}
\]

where \(I_f\) and \(I_b\) are the intensity of foreground and background respectively.
The spatial resolution of the image is the smallest distance between two parallel lines or points that can be resolved as two different entities (Stock, 1999). In 3D images, the resolution is usually approximated by voxel size, which is the size of the minimum regular grid in the image fields. Recently, commercial nano-CT systems were introduced to research laboratories, and the spatial resolution claimed are substantially below 1 µm (Stock, 2008). From the point view of resolution, micro-CT is superior to other non-destructive techniques such as ultrasound and magnetic resonance imaging (MRI), with typical resolutions of 30 µm and 100 µm respectively (Bolland et al., 2008).

Due to imperfection of the system components and the assumptions used for reconstruction, there are also artefacts in micro-CT images, such as beam hardening and ring artefacts.

Beam hardening artefacts occur because X-rays with different energies have different adsorption rates: lower energy photons get absorbed more compared to the higher energy photons (Stock, 1999). However, the reconstruction algorithm assumes that a monochromatic beam is used. Therefore, the edge of a thick object would have higher attenuation coefficient due to the low energetic X-ray it absorbed. This effect is also responsible for the star effect of highly attenuating matter surrounded by low attenuating neighbours.

![Figure 2.4. Influence of the beam hardening correction. (a) Cross-section through a sample holder (light blue) filled with a homogeneous $K_2HPO_4$ solution of 500 mg/cm$^3$, scanned with low radiation energy without beam hardening correction; (b) same as (a), but with implementation of a polynomial correction algorithm; (c) line profiles through the cross-sections of (a) and (b) (black bars); In (c), blue line indicates the theoretical value; black curve is the uncorrected profile; and red curve is the corrected profile. Modified from (Mulder et al., 2004).](image)

For example, the beam hardening artefact and a correction result are demonstrated in Figure 2.4; Mulder et al. using $K_2HPO_4$ solution, which has the same absorption characteristics as hydroxyapatite over a wide range of energies, as the reference material to obtain the beam
hardening profile for polynomial correction (Mulder et al., 2004, Mulder et al., 2006). In an ideal case, the K$_2$HPO$_4$ solution region in the micro-CT image should possess identical voxel intensity. However, due to the beam hardening effect, the edge of the homogeneous K$_2$HPO$_4$ solution has a much higher attenuation compared to the central region (as shown in Figure 2.4a). Once the non-linear beam hardening profile has been obtained, it can then be used to numerically correct the voxel intensities in the micro-CT scan of the material with similar X-ray adsorption characteristics. Another common way to reduce beam hardening effect is to use a thin layer of Cu or Al to block the low energy photons before it penetrating the sample.

Ring artefacts (Figure 2.5) are caused by imperfections in the detector, i.e. variations in the response of individual pixel-elements. When translating the attenuated X-ray into numbers, an imperfect detector element may consistently vary (either increase or decrease) the intensity gain during the imaging process. This artefact would cause a horizontal line in the sinogram (Figure 2.5d) and therefore a concentric ring is superimposed on the reconstructed image (Figure 2.5c). This effect needs to be corrected before data visualisation and quantification.

![Figure 2.5. Effect of ring artefact: (a) a simulated phantom; (b) the sinogram of (a) by parallel projection; (c) and (d) similar as (a) and (b), show the effect of ring artefact on the reconstructed image and sinogram respectively.](image)

Artefacts can be reduced by incorporating dark-field and flat-field scans into the imaging processing (Titarenko et al., 2010b). Dark-field are blank scans without X-ray beam, and flat-field scans use the X-ray energy setting for sample scanning, but without a sample. The gain
profile of the detector can be obtained and normalised by these two scans, and then used as an offset to reduce the artefacts.

Ring artefacts can be significantly reduced by introducing jittering during the scan (Davis and Elliott, 1997). Jittering means during the scan, the sample stage is not only rotated, but also has small random displacements in one, or both, the X and Y directions (perpendicular to X-ray beam). The displacement profile is recorded so that it can be used to generate appropriate sinogram from radiographs. The effect of imperfect detector elements is averaged out by the jittering process, significantly reducing ring artefacts.

Ring artefacts can also be reduced by image processing on the sinogram (Titarenko et al., 2010a). The imperfect detector element would cause a horizontal line (parallel to the rotating degree axis) in the sinogram. The line can be eliminated to reduce the ring artefact in the final reconstruction.

Finally, the slice in reconstructed volume which containing ring artefact can be transform into polar coordinates, so that the ring was transform to a vertical line. This vertical line can be eliminated by image processing before reverse transform back to Cartesian coordinate system (Sijbers and Postnov, 2004).

2.4. Scaffold quantification via micro-CT

Due to its advantageous non-destructive property and high resolution, micro-CT has been intensively used for hard tissue imaging after Feldkamp et al., who firstly used micro-CT to study the 3D structure of human trabecular bone in 1989 (Feldkamp et al., 1989). Subsequently, micro-CT has also been a popular tool for bone scaffold characterisation (Guldberg et al., 2003, Cartmell et al., 2004), especially ceramic based scaffolds which are with a suitable range of mass attenuation coefficients.

Early studies used micro-CT primarily as an observation tool to visualise the structure of bone and/or scaffold qualitatively. Quantification methods were based on 2D slices and were similar to those applied on SEM images. Recently, novel 3D image processing based algorithms have been developed for porous structure quantification, which can quantify the pore network thoroughly for optimal scaffold design in bone tissue engineering (Jones et al.,...
Watershed transform, skeletonisation, and accessible radius are popular algorithms for pore network segmentation.

Atwood et al. (Atwood et al., 2004) first used a watershed based method to quantify a scaffold in a semi-automated fashion. After a distance transform and watershed transform (Mangan and Whitaker, 1999), an in-house code was developed to locate the interconnects. From the segmentation, individual pores and interconnects in the pore network were identified. Therefore not only the distributions of pore or interconnect sizes in the entire sample, but also the individual pore or interconnect size and shape were obtained.

However, the approach by Atwood et al. had several known issues: 1) the interconnect size was represented using the diagonal length of a coordinate bounding box, which gives the upper limit of the interconnect size, effectively an over-estimate; 2) Any voxels identified as being in a pore that had a neighbouring voxel belonging to a different pore, were grouped to form interconnects. If two pores were connected with multiple interconnects, the programme recognised them as a single pore, which also significantly over estimated the interconnect sizes. The same authors (Jones et al., 2007c) introduced a geometrical correction factor which was determined by using manual measurements of individual interconnects to reduce the overestimation caused by the bounding box method. However, the geometrical correction factor method provides only an average correction, producing a large local error if the pore or interconnect shape is far from the average shape.

Sol-gel derived bioactive glass foams were quantified by Atwood’s method and results shows that the glass foam has suitable pore and interconnects size as a bone regeneration scaffold (Atwood et al., 2004). The accessibility of the pore in the scaffold was first time quantified by means of interconnect size. Jones et al. (Jones et al., 2007c) also demonstrated that micro-CT can help to optimise the bioactive glass foam design by determining the better sintering temperature. They found that the 70S30C bioactive glass foam can be sintered to 800 °C to gain mechanical strength, as well as remain suitable pore structure and bioactivity.

Many other research groups also used micro-CT for scaffold quantification on various tissue scaffolds (Jones et al., 2007a, Moore et al., 2004, Otsuki et al., 2006, Otsuki et al., 2007, Jones et al., 2004, Jones et al., 2009a). Jones and co-workers at Australian National University carried out several studies on scaffold quantifications from micro-CT images (Jones et al., 2009a, Jones et al., 2004). They looked at the accessibility of the pore structure,
and investigated the bone ingrowth rate in HA scaffolds. In their recent work (Jones et al., 2009a), the segmentation of the 3D images was based on watershed transform guided by the skeleton obtained by medial-axis transform. Many others also use medial axis transform or topology skeleton to represent the porous structure of scaffolds.

Soft tissue or blood vessels are difficult to be segmented and quantified from micro-CT images when scanning together with bone or scaffold due to their relatively low X-ray attenuation coefficients (Figure 2.2). Many studies (Bolland et al., 2008, Plouraboue et al., 2004, Duvall et al., 2004, Heinzer et al., 2006, Malyar et al., 2004, Moore et al., 2003, Lu et al., 2006) use contrast enhanced micro-CT to tackle this issue. For example, Bolland et al. (Bolland et al., 2008) use radio-opaque dye to evaluate the neovascularisation in ex vivo tissue engineered bone constructs with micro-CT.

Other imaging methods, such as phase contrast tomography (Momose et al., 1996) and magnetic resonance imaging (MRI), are more appropriate in directly imaging samples which consist of soft tissue (or low X-ray attenuation material). Cloetens et al. demonstrates that the use of phase contrast tomography can provide clear edge separation, as well as quantitative information when coupled with adsorption micro-CT (Cloetens et al., 2003). However, micro-CT is more appropriate for this project as the materials have high X-ray attenuation.

Besides quantification, micro-CT images can also be converted into finite element models for finite element analysis or computational fluid dynamic simulation. Permeability simulation is one the most popular simulations which can be derived from 3D image (Jones et al., 2007c, Jones et al., 2009a). Mechanical properties of the scaffold can also be predicted through simulations, as demonstrated in (Jones et al., 2009a).

2.5. Bioactive glasses as bone scaffolds

At present, a scaffold that fulfils all of the design criteria listed in section 2.2 does not exist. Bioactive glasses, which are amorphous, silicate-based materials, are good candidate materials for tissue engineering scaffold fabrication due to their unique bioactive properties which can be tailored depending on the composition (Hench, 1998).

The first generation of bioactive glass – the melt-derived 45S5 (46.1 mol.% SiO2, 24.4 mol.% Na2O, 26.9 mol.% CaO and 2.6 mol.% P2O5) Bioglass® was invented by Hench in 1971
(Hench et al., 1971). Bioglass® is the first man-made material that can achieve direct bonding to living tissue (Hench et al., 1971, Hench and Paschall, 1973); and it can also promote bone growth (Hench, 1991, Xynos et al., 2000, Xynos et al., 2001) at the genetic level. Bioglass® has been clinically used as a treatment for periodontal disease PerioGlas® (Fetner et al., 1994), a bone filling material Novabone® (Hench, 1991), and also been used to replace damaged middle ear bones (Wilson et al., 1995). Recently, Hench reviewed the invention, synthesis, properties characterisation and commercialisation of Bioglass® (Hench, 2006).

Besides Bioglass®, other glasses compositions have been developed and been found to be bioactive, as listed in Table 2-1. In general, they are silica-based glasses that were altered with a number of network modifiers such as Ca, Na, and P. However, there are two methods for synthesising the glasses: the traditional melt-derived route and the sol-gel chemistry approach.

Table 2-1. Typical compositions of silica based bioactive glasses, in molar percentage.

<table>
<thead>
<tr>
<th>Glass Type</th>
<th>SiO$_2$ (mol.%)</th>
<th>CaO (mol.%)</th>
<th>Na$_2$O (mol.%)</th>
<th>P$_2$O$_5$ (mol.%)</th>
<th>SrO (mol.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>45S5 Bioglass®</td>
<td>46.1</td>
<td>26.9</td>
<td>24.4</td>
<td>2.6</td>
<td>-</td>
</tr>
<tr>
<td>58S (sol-gel)</td>
<td>60</td>
<td>36</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>70S30C (sol-gel)</td>
<td>70</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>StronBone®</td>
<td>44.47</td>
<td>21.47</td>
<td>27.26</td>
<td>4.42</td>
<td>2.39</td>
</tr>
</tbody>
</table>

The bonding between bioactive glass and living tissue is achieved through the formation of a hydroxycarbonate apatite (HCA) layer on the glass surface after contract with body fluid (Hench et al., 1971, Jones et al., 2007b); the HCA is chemically similar to the apatite in natural bone. Figure 2.6 shows the 10-stage mechanism that has been developed by Hench to explain the bonding of bioactive glasses to bone (Hench, 1991). The formation of the HCA layer is a result of a sequence of chemical reactions on the surface of the bioactive glass after implantation (stages 1 – 4). After the corrosion of soda-lime-silica glass, biological mechanisms of bonding are then thought to follow, which involves the adsorption of growth factors, attachment, proliferation and differentiation of osteoprogenitor cells (Hench and Polak, 2002). However stages 5 – 10 are not yet proven. A nano composite of mineral and
collagen on the surface of the implants is formed by mineralised extracellular matrix (ECM, a collagen matrix) laid down by osteoblasts (Ducheyne and Qiu, 1999).

Figure 2.6. Schematic of the 10-stage reaction mechanism for the bonding of bioactive glasses to bone. Modified from (Hench, 1991) and (Jones et al., 2001).

Traditional bioactive glasses are manufactured by the melt-derived approach and are often in the form of powders, particles, granulates, or dense bulk (monolith) of various shapes and sizes. Bioactive glasses can also be fabricated by the sol-gel process.

The sol-gel technique (Hench and West, 1990) involves a transition from sol (a fluid suspension of colloidal particles) to gel (the colloidal particles aggregate and form a non-fluidic network through the condensation reaction), and finally to glass after appropriate thermal treatment. The sol-gel fabrication process consists of five stages, which are mixing,
casting, ageing, drying and stabilisation. For example, when making bioactive glass with binary 70S30C composition, sol preparation involved the hydrolysis of tetraethyl orthosilicate (TEOS) (the silica precursor). Calcium was added to the composition using calcium nitrate. Hydrolysis of the TEOS creates silanol (Si-OH) groups. A Si-O-Si bond can be formed by condensation of two silanol groups; and gelling was achieved when the inorganic network has been built up throughout the solution.

The bioactive glasses made from sol-gel route are more bioactive due to an inherent nanoporosity, higher surface area and more rapid dissolution compared to melt-derived glasses with similar composition. Although the compositional inhomogeneity in sol-gel glasses is thought to be reduced compared to melt-derived glasses, it can still be observed. By using SEM with EDX, Saravanapavan and Hench found that calcium concentration was higher at the edge of the cylindrical monoliths of 70S30C composition (Saravanapavan and Hench, 2003). A calcium distribution was also observed by Yue et al from synchrotron micro-CT images of the 70S30C foam scaffolds (Yue et al., 2010).

In 1991, Li et al. developed the first sol-gel derived 58S (60 mol.% SiO₂, 36 mol.% CaO, 4 mol.% P₂O₅) bioactive glass (Li et al., 1991). Melt-derived glass are not bioactive if the SiO₂ content is greater than 60 mol.% but sol-gel derived glasses can be bioactive while containing up to 80 mol.% SiO₂. Sol-gel derived glasses have enhanced bioactivity because of their nanoporosity (Jones, 2009). The surface area to volume ratio of sol-gel derived bioactive glasses are two orders of magnitude higher than those for melt-derived bioactive glasses of the same composition (Hench and West, 1996, Jones et al., 2001). For example, the sol-gel derived bioactive glass with 70 mol.% of silica (70S30C) can rapidly form a HCA layer in vitro (Li et al., 1991, Greenspan et al., 1994, Pereira et al., 1995, Pereira and Hench, 1996). The reason that 70S30C glass can form a HCA layer even though it does not contain P can be attributed to Si-OH groups which form during the corrosion of the glass. There are several Si-OH groups present in the un-reacted sol-gel derived glasses that can act as nucleation sites. In addition, the sol-gel process is initially a room temperature process, making it possible to fabricate hybrid materials (Poologasundarampillai et al., 2010), where the polymer and the silica interact at the molecular level (Mahony et al., 2010).

It is not possible to produce porous glass scaffolds from the original Bioglass® composition because the glass crystallises during sintering. So perhaps the greatest benefit of the sol-gel method is that macro porous bioactive glass foams can be produced (Sepulveda et al., 2002a,
Saravanapavan and Hench, 2003), which are good bone scaffold candidates. SEM images (Figure 2.7) show several sol-gel produced bioactive glass foams with interconnected macro porous networks.

Figure 2.7. SEM images of (a) 100S, (b) 70S30C, and (c) 58S foams after thermal stabilisation at 600 °C. Modified from (Sepulveda et al., 2002a).

Figure 2.8 is the schematic flow chart for the processing of bioactive glass foams. The macro pores are achieved by agitating vigorously before gelation to trap air bubbles in the sol. One key aspect to making bioactive glass with a macro porous structure is that the gelation time is reduced from three days to several minutes by introducing hydrofluoric acid (HF), an effective catalyst (Sepulveda et al., 2002a). HF can be removed later during the thermal stabilisation treatment. During sintering, the scaffold shrinks and the connecting regions between pores will open up to form interconnects (Jones et al., 2006b). The foaming process has been optimised by Jones et al. by adjusting factors like foaming temperature, surfactant, water fraction (the $R$ ratio), and sintering temperature (Jones et al., 2006a, Jones et al., 2007c). A detailed foaming process is presented in section 4.1.1.

Figure 2.8. Flow chart of the sol-gel foaming process. After (Jones et al., 2010).
The hierarchical porous structure of 70S30C bioactive glass foam is very similar (Jones et al., 2007c) to that of human trabecular bone as shown in micro-CT images (Figure 2.9). Previous characterisation results (Sepulveda et al., 2002a, Jones and Hench, 2003a, Jones and Hench, 2004, Jones et al., 2006a, Jones et al., 2007c) by SEM, MIP, and micro-CT both suggest that bioactive glass foams can be fabricated with suitable sizes of macro pores with equivalent diameters around 600 µm and interconnect diameters around 200 µm (as shown in Figure 2.10). The MIP result is obtained from the constrictions on the flow of mercury into the scaffolds as a function of pressure applied (obtained from the Washburn equation). It is not possible to be sure exactly what constrictions are being measured or the shape of the interconnects that are being measured. MIP is also a destructive technique. Resolution limitations of the micro-CT may also contribute to the MIP values being lower.

Figure 2.9. Micro-CT images of (a) a typical 70S30C bioactive glass scaffold produced by the sol-gel foaming process; and (b) human trabecular bone.

Figure 2.10. Interconnect size distributions of a 70S30C foam scaffold obtained by Mercury Intrusion Porosimetry (MIP, left y-axis) and image analysis of micro-CT images (number density, right y-axis). Modified from (Jones et al., 2010).
2.5.1. Dissolution studies of bioactive glasses

A synthetic bone graft is expected to bond with the host bone to enable stable fixation and to degrade at the same rate of the new bone formation. It should also share load with the host bone. The HCA layer formation rate is an important factor to evaluate the bioactivity of the material in vivo (Hench, 1991). Slow HCA formation can lead to fibrous tissues forming surrounding the glass and the material becomes inert rather than bioactive. On the other hand, if a scaffold dissolves too quickly to allow HCA deposition, its mechanical strength degenerates before new bone is formed to take the load. The formation of HCA layer in vivo is extremely complicated and can be affected by both chemical and physical conditions. For example, parameters like pH value, ion concentrations, surface to volume ratio of the scaffold, are inherently linked and both have effects on the dissolution rate and bone bonding ability of the scaffold in vivo (Sepulveda et al., 2000, Saravanapavan and Hench, 2001). Simplified methods such as a dissolution experiment are used to study the bioactivity of materials in vitro. Jones et al. investigated the effect of local ion concentrations on HCA formation of bioactive glass powder in simulated body fluid (SBF) (Jones et al., 2001). They found that the concentration of glass in the SBF was critical. HCA forms at relatively low levels of Ca ion dissolution by deposition of amorphous calcium phosphate, which then crystallises to form HCA. As glass concentration increased, calcite deposited as there was more Ca ions present in the SBF than phosphate species so carbonate ions reacted with excess the Ca (Jones et al., 2001). This suggests that the bioactivity of bioactive glasses in vivo can be maximised or manipulated by controlling the dissolution rate. Sepulveda et al. points out that the active ion release rate of bioactive glass particles can be controlled by surface area, e.g. by particle size range, glass type, and powder volume fraction (Sepulveda et al., 2002b).

Dissolution is not only important for HCA formation but also for rate of delivery of active ions to cells. The ion concentrations affect the new bone regeneration ability in vitro and in vivo (Hench, 2009, Valerio et al., 2004). Dissolution products from bioactive glass enhance bone regeneration by interacting with cell genetic systems. Xynos et al. shows that the released products of Bioglass® can activate and upregulate the gene-expression profile of human osteoblasts using cDNA microarray analysis (Xynos et al., 2001). Soluble Ca ions and Si species need to be kept at a particular ratio and suitable ranges of concentrations. For example, the suitable concentrations for Ca and Si species are 60 – 90 ppm and 15 – 30 ppm respectively (Hench, 2009). In addition, the optimum activation Si ions concentration of...
approximately 20 μg/ml of soluble Si per 20,000 cells has been identified (Xynos et al., 2001). Bielby et al. demonstrated that bioactive glass dissolution products can enhance the derivation of osteoblasts from pluripotent human embryonic stem cells via analysing the capacity to form mineralised tissue both in vitro and in vivo (Bielby et al., 2004). Therefore, it is important to control the dissolution rate of the bioactive glasses from an osteostimulation point of view. A recent review by Hench has showed the interaction between degradation ions of different types of bioactive glasses and osteoprogenitor cells (Hench, 2009). Besides of genetic effects, Si species and Ca ions released by bioactive glasses can provide resource for new bone mineralisation and formation.

Simulated body fluid (SBF), which has ion concentrations nearly equal (Table 2-2) to those in human blood plasma, is a popular choice to carry out in vitro dissolution studies (Kokubo and Takadama, 2006). Kokubo, the inventor of the original SBF, also proposed a method to test the bioactivity of material in vitro using SBF (Kokubo et al., 1990b), based on the fact that a number of bio ceramics can form surface HCA layers after immersion in SBF (Kokubo et al., 1990a, Kokubo et al., 1990b).

Table 2-2. Ion concentrations of SBFs and human blood plasma. After (Kokubo and Takadama, 2006).

<table>
<thead>
<tr>
<th>Ion concentration (mM)</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Mg²⁺</th>
<th>Ca²⁺</th>
<th>Cl⁻</th>
<th>HCO₃⁻</th>
<th>HPO₄²⁻</th>
<th>SO₄²⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human blood plasma (Gamble, 1954)</td>
<td>142.0</td>
<td>5.0</td>
<td>1.5</td>
<td>2.5</td>
<td>103.0</td>
<td>27.0</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Original SBF (Kokubo et al., 1990b, Filgueiras et al., 1993)</td>
<td>142.0</td>
<td>5.0</td>
<td>1.5</td>
<td>2.5</td>
<td>148.8</td>
<td>4.2</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Corrected SBF(c-SBF) (Ohtsuki et al., 1991, Kokubo, 1991)</td>
<td>142.0</td>
<td>5.0</td>
<td>1.5</td>
<td>2.5</td>
<td>147.8</td>
<td>4.2</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Revised SBF (r-SBF) (Oyane et al., 2003)</td>
<td>142.0</td>
<td>5.0</td>
<td>1.5</td>
<td>2.5</td>
<td>103.0</td>
<td>27.0</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Newly improved SBF (n-SBF) (Takadama et al., 2004)</td>
<td>142.0</td>
<td>5.0</td>
<td>1.5</td>
<td>2.5</td>
<td>103.0</td>
<td>4.2</td>
<td>1.0</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Recently, the correlation between HCA formation rate in static SBF solution and material’s *in vivo* bone bonding ability has been questioned by many researchers (Bohner and Lemaitre, 2009, Pan et al., 2010). The traditional SBF based bioactivity test does not incorporate the CO$_2$ partial pressure into the system and which is important in the physiological environment. Additionally, the SBF system may provide false prediction of material’s apatite formation ability *in vivo*.

Glass powder or monoliths in a static medium (*i.e.* SBF) was used in previous studies to investigate the chemical influence of the materials. However, the human body is a dynamic environment and fluid flow affects ion transport away from a surface and deposition of species on the surface. 3D porous scaffolds behave differently from powders or monoliths because of local variations in flow rate, pressure, and ion concentrations due to compositional inhomogeneity and preferential flow.

One way to better mimic the dynamic environment in the body is to use a bioreactor, which can provide and monitor changes in biological, chemical or physical environmental factors in a reproducible and controlled manner (Martin et al., 2004, Pörtner et al., 2005). There are different types of bioreactors commonly used in bone tissue engineering applications, such as spinner flask, rotating wall vessel, and perfusion reactors (Bancroft et al., 2003).

In Chapter 4 of this thesis, a simple perfusion bioreactor was used to ensure flow of SBF through a porous scaffold. The impact of perfusion flow on the 70S30C foam scaffold dissolution properties was studied. Compared to static dissolution experiment, the perfusion bioreactor can provide continues flow with controlled flow rate, mechanical stimuli (shear), as well as better solute exchange in the scaffold (David et al., 2010).

When the cell culture is involved, the use of bioreactor may be critical in tissue engineering applications as they can offer monitored and controlled environmental and operation conditions for cell growth. The bioreactor design for tissue engineering is complex and needs to be tissue specific.

### 2.6. Ti scaffold fabrication by selective laser melting

Titanium (Ti) and its alloys has been a material of choice for load bearing implants for orthopaedic and dental applications due to its excellent biocompatibility, mechanical strength,
wear resistance, and corrosion resistance (Kienapfel et al., 1999). One major issue with such implants is establishing permanent fixation to bone after surgery (Bobyn et al., 1980). One accepted method to improve fixation is the addition of texturing to the implant surface providing mechanical interlocking of the implant and bone after bone ingrowth has occurred. Pilliar et al. (Pilliar et al., 1975) developed one of the first techniques in 1975 to produce a surface texture for interlocking by sintering large (>100 μm) Ti particles onto the surface of the implant creating porosity for bone ingrowth. Since this early work, a range of different pore networks and surface treatments have been developed to encourage osteoinduction and bone ingrowth; improving fixation (Pattanayak et al., 2011, Kienapfel et al., 1999).

However, even with good fixation, having a Young’s Modulus higher than bone can cause stress shielding. One method of reducing the effective modulus of the implant is to make it porous throughout its structure such that it reduces the effective moduli to better match the host bone (Thieme et al., 2001, Ryan et al., 2006, Krishna et al., 2007). Producing such structures with open porosity can be achieved via a variety of methods, as reviewed by Singh and Lee (Singh et al., 2010). One of the most promising techniques is Selective Laser Melting (SLM), an additive manufacturing technique which allows complex three dimensional (3D) structures of Ti to be built accurately from computer models (Wehmöller et al., 2005, Vandenbroucke and Kruth, 2007, Mullen et al., 2009). In a typical SLM building process, the component can be built additively in a layer-wise fashion: the Ti powders are applied in thin sequential layers onto a substrate plate (i.e. powder bed method) and then selectively melted (according to a computer design) using a scanning laser beam (typically powers of 200 W, a 60 micron spot size and 0.5m/s speed). The accuracy of SLM is usually better than tens of microns; depending on the operational conditions and Ti powder particle size (Gibson et al., 2009, Yadroitsev et al., 2007).

2.7. Objectives of this thesis

The primary objective of this thesis was the development of image processing based method for pore network quantification from three dimensional micro-CT images of tissue scaffolds.

Image segmentation is one of the oldest and most widely studied problems in digital image processing. However, due to the complexity of 3D porous structures, a method which can work for all different types of porous scaffolds does not exist. Additionally, even a method
that has been showed successful to quantify a particular type of scaffolds; it may still need to be heavily tailored to the characteristics of a specific data set. Therefore, the objectives of this thesis are as follows:

- Improve the procedures for bioactive glass foam scaffolds quantification in terms of thresholding, region of interest selection, segmentation, and feature measurement.
- Develop a method for non-destructively investigating the degradation and bioactivity of bioactive glass foam scaffolds in a perfusion flow environment.
- Adapt the quantification methods for completely different structures of Ti scaffolds produced by selective laser melting (SLM).
- Develop methods such as surface roughness quantification which can act as quality control and quality assurance tools for SLM built Ti scaffolds.
3. **QUANTIFICATION OF BIOACTIVE GLASS SCAFFOLDS WITH MICRO-CT**

Bone scaffolds are expected to act as templates to guide bone ingrowth and tissue regeneration *in vitro* and *in vivo*. To enable bone ingrowth, mass transport, cell migration, and vascularisation, a scaffold with an interconnected porous structure is essential. Bioactive glasses, which are amorphous, silica-based bioactive materials, can be fabricated into three dimensional foams with preferable pore and interconnect size distributions, therefore they are good candidate scaffolds for bone tissue engineering.

Micro-CT imaging is non-invasive and non-destructive; and can provide a 3D X-ray attenuation map of the object. The X-ray attenuation is closely related to the density of the material. Therefore, the structure and composition of the scaffold can be visualised, and quantified, making micro-CT a powerful and popular tool for scaffold characterisation.

With micro-CT and 3D image analysis algorithms, bioactive glass foam scaffolds can be evaluated quantitatively. Structural parameters that closely relate to the performance of the 3D scaffold, such as porosity, pore and interconnect size distributions, can be quantified non-destructively from micro-CT. In this chapter, imaging and quantification strategies are discussed for bioactive glass scaffold characterisation.

### 3.1. Imaging the scaffold

Bioactive glasses, primarily consisting of silica and calcium species, are well suited to providing high-contrast micro-CT images from both laboratory based and synchrotron

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radiation facilities. Because micro-CT scanning is non-destructive, outside of ensuring the sample is in the field-of-view throughout the rotatory imaging process, there are no special requirements for sample preparation. However, in practice, cylindrical samples are preferred and the vertical axis of the sample can be set to be perpendicular to the X-ray beam and rotating plane (Ketcham and Carlson, 2001). Samples with other shapes can be placed into a cylindrical sample holder made of X-ray transparent material (or low X-ray attenuating material), which can then be easily centred on the sample stage to reduce the chance of sample moving during scanning.

In order to reduce beam hardening artefacts when using a polychromatic X-ray source, physical filtering is normally applied to pre-harden the X-ray beam. A thin layer (hundreds of microns thick) of Al or Cu can be placed between the sample and X-ray source, also perpendicular to the X-ray beam. In this way, lower energy X-rays are filtered out so that the effective X-ray spectrum is shifted to higher energies (hardened). However, longer exposure times are needed to achieve high signal-to-noise level, and the contrast resolution may reduce due to harder effective spectrum.

Reference material, or a reference wedge, with a similar X-ray attenuation to the sample, can also be imaged at the same time and used to correct the beam hardening artefact; it is also useful to normalise the CT values among different scans (Ketcham and Carlson, 2001). For example, a cylindrical wedge made of homogeneous material that is similar (in terms of atomic number and density) to the sample can be scanned at the same condition of the sample to obtain the beam hardening profile. Thereby the micro-CT image of the interested sample can be corrected afterwards by image processing techniques, such as inverse polynomial correction (Mulder et al., 2004).

In addition, two types of wedge materials, one made with low attenuating material and one made with a similar material to the samples, can be used to normalise voxel intensity levels among several scans.

To achieve high contrast images of biological samples, micro-CT scans with lower X-ray energy and longer exposure times are used. Although the bioactive glass scaffolds alone can be sharply imaged with an X-ray beam with a range of peak energies, when inter-scan comparison is needed, for example comparing the same scaffold before and after implantation, a lower X-ray energy level is preferred. In this way the ex vivo samples
consisting of a degraded scaffold, bone (both fully and partially mineralised), and soft tissue can be better imaged at low energy in terms of contrast resolution.

3.2. Pre-processing

In order to improve the accuracy of the quantification, initial data processing is necessary. The common techniques are described below.

3.2.1. Intensity normalisation

If the same reference materials are used for several micro-CT scans, the images can be normalised to same intensity level with Equation 3.1. The voxel intensity $I$ in the new image is:

$$I = \frac{I_{\text{original}} - I_{\text{low}}}{I_{\text{high}} - I_{\text{low}}}$$  \hspace{1cm} 3.1

Where $I_{\text{original}}$ is the original intensity, $I_{\text{low}}$ is the average intensity of the low attenuating material, and $I_{\text{high}}$ is the average intensity of the high attenuating material.

Figure 3.1. Positions of the reference materials (Teflon and glass fibre) in a scan of a bioactive glass scaffold as (a) 3D rendering and (b) 2D slice.

For example, a piece of silica glass fibre and a Teflon sample holder were used as reference materials to normalise the scans of a 70S30C foam scaffold scanned at different stages of dissolution in an SBF flow environment (detailed methodology can be found in Chapter 4).
The silica glass fibre can be segmented from the images by a Connected Component Labelling Algorithm (Haralick and Shapiro, 1992). The Teflon ring in the image had low average intensity, and can be extracted based on the relative location to the scaffold. The segmentation of the reference materials for the images is illustrated in Figure 3.1.

After successful segmentation, the two reference phases, $I_{low}$ and $I_{high}$ can be calculated accordingly and used to normalise the scans to each other. In Figure 3.2a, the histograms of the two reference materials in micro-CT images taken at different dissolution time stage were compared before normalisation. The peaks with smaller modes represented the Teflon phase, and the peaks with greater modal values were the glass fibre phase. Although the position of Teflon phase remained almost unchanged for all four scans, the modal value of the glass fibre phase distribution was much smaller in the initial 0 h scan compared to which in later scans. The histogram of two reference materials showed varying intensity levels among different scans although they were assumed to not react with the SBF flow. If the assumption was made that the CT values were correlated to material density, the raw images without normalisation would lead to false observation of scaffold degradation and redeposition. In

![Graphs](image)
Figure 3.2b, the histograms of reference materials in normalised images showed aligned modal values for both Teflon phase and glass fibre phase among four scans. Although this linear transition (basically an *offset and gain* operation) of voxel intensity may not perfectly fit the true profile of the CT value versus physical density distribution, the result interpreted from normalised images is much more appropriate.

### 3.2.2. Image filtering

Computed tomography data is frequently very noisy due to the relatively low flux, the imperfections in the imaging chain and discretisation during volume reconstruction. Median filtering is normally applied to reduce noise level of micro-CT images whilst preserving edges in the image.

Many other image filters are often applied when performing FBP, usually in Fourier space (frequency domain). Filters can be classified as low-pass filters or high pass-filters according to their frequency response properties. Low-pass filters are usually applied to reduce noise and high-pass filters are usually used to enhance edge or image contrast (Stauber and Müller, 2008). A common filter for image processing is a Gaussian filter (Figure 3.3 c & d). The Gaussian function is symmetric and separable in multidimensional operation. More importantly, several sequential Gaussian filtering can be replaced by another single Gaussian filtering. Multiple Gaussian based operations, such as noise reduction and gradient calculation, can be combined into a one-pass filtering process. Another important filter is median filter (Figure 3.3 e & f), which is commonly used to reduce the Salt and Pepper noise in the image.

More advanced filters are also used to threshold the final volume image in Cartesian space. For example, anisotropic diffusion filtering (Perona and Malik, 1990) can preserve edge as well as smoothing the low frequency region of the image.
3.2.3. Thresholding

Thresholding is a basic but important step in quantification, dividing the original image into different labelled groups according to voxel intensities and sometimes spatial dependence. If there are two phases (for example, scaffold phase and background phase) that need to be separated, the process is also called *binarisation*. The threshold value strongly influences the calculation accuracy of porosity of the scaffold.

To binarise a scaffold scan with high contrast, a simple global threshold can be used. The threshold value was chosen from histogram of the image, equidistant between the two peaks of background phase and scaffold phase (Figure 3.4). There are also automated thresholding methods which are based on the histogram operations with different measuring functions. For
example, the Otsu method (Otsu, 1975), which chooses the threshold so that it minimise the within-class variance.

Figure 3.4. Example of global thresholding: (a) and (b) are micro-CT images of melt-derived ICIE.16M bioactive glass before and after global thresholding respectively. (c) is the histogram of the image. The threshold was selected as equidistant between the background peak and scaffold peak in the histogram.

Sometimes the images contrast is poor, and the peaks in the histogram are not distinct. For example, in micro-CT scans of scaffolds after \textit{in vitro} culture or implantation both scaffold degradation and bone tissue regeneration can contribute to “blur” the boundary between phases. In these scenarios, local thresholding methods are needed to separate different phases in the image.

In local threshold methods, a smaller volume window is sliding over the entire image. In each sub volume, the threshold can be chosen in a similar fashion to a global threshold, but based on the local histogram. There are also more complicated algorithms, where the spatial dependence is considered to best “predict” the phase of the boundary voxels, such as the Indicator Kriging method (Oh and Lindquist, 1999).
3.2.4. Region of interest selection

A region of interest (ROI) is a term for a sub volume of the image which contains features for analysis. For example, the bulk region of the scaffold phase can be extracted by morphological operations (dilation, erosion, or combination of the two) or convolution based methods.

Also, to reduce the chance of false segmentation, small unconnected segments were normally removed using a Connected Component Labelling algorithm or Burn algorithm (Stauber and Müller, 2008, Haralick and Shapiro, 1992).

3.3. Segmentation

For bioactive glass foams with interconnected macro pores, a watershed transform with a height function of distance map has been shown to be an effective strategy to segment the pore network (Atwood et al., 2004, Jones et al., 2007c, Yue et al., 2010, Karakoti et al., 2010, Wu et al., 2011, Yue et al., 2011).

A typical procedure to segment the pore space (background phase) from a binarised image (Figure 3.5a) is first to apply a distance transform and then invert the obtained distance map (so that the pore centres are minima). Voxel values of the scaffold phase were also set to be equal to the global minimum so that it was segmented as a group of struts. The modified distance map (Figure 3.5b) can be viewed as a topographical map (Figure 3.5c). Finally the watershed transform is applied to separate the void space voxels into groups (Figure 3.5d), which repent macro pores in the pore space.

The watershed transform (Beucher and Lantuéjoul, 1979, Meyer, 1994), as the name suggests, groups the voxels from the height map into catchment basins which were separated with watershed lines. Figure 3.6a shows a representative piece of height function which has three regional minima and four regional maxima. The watershed algorithm considers the height map a topographical relief being flooded. Voxels, from which the virtual water flows to the same local minimum (i.e. the bottom of a catchment basin) are grouped. The segmented groups are separated at watershed lines, where flow can go to either of the two adjacent catchment basins. For example, the height function shown in Figure 3.6a was segmented into three catchment basins by a watershed transform, as shown in Figure 3.6b.
Figure 3.5. Procedure for scaffold segmentation from a micro-CT image. (a) A binarised image can be transformed into a (b) modified distance map, which can be viewed as a (c) topographical map. The modified distance map was segmented into (d) different pores by watershed transform, labelled with colours.

Figure 3.6. A schematic of (a) a height map and (b) segmentation following application of the watershed transform.
However, when applying the technique to a real dataset, significant over segmentation frequently occurs at a multitude of local minima from surface roughness or noise. Therefore, filtering is normally required.

To overcome this problem, smoothing and merging strategies can be used. Spatial smoothing can eliminate small topographical variations in the height map, reducing the total number of segmented groups. Smoothing can be achieved by filtering, morphological operation, or statistically based methods. However, filtering sometimes affects the location of the larger topographical variations in the height map as well; therefore the level of smoothing must be carefully controlled. Over smoothing can effectively reduce the resolution of the image. Merging strategies apply rules to selectively join shallow regions to their neighbours. The insignificant regions can be selected by measuring the within-region difference from the height map (Sijbers et al., 1997, Mangan and Whitaker, 1999).

The filtered grayscale image can be directly used as a height map for the watershed transform (Sijbers et al., 1997). For bioactive scaffold quantification, a distance map derived from a binarised micro-CT scan is commonly used. Atwood et al. (Atwood et al., 2004) used a dilation based method to transform binary image of scaffold into 3D distance map. The scaffold phase was dilated step by step, until all the voxels belonged to background phase were replaced. The number of steps required to replace each background voxels is the value of that voxel in distance map. Other distance transform schemes use different metrics, such as a Euclidian metric (Maurer Jr et al., 2003), City Block metric (Rosenfeld and Pfaltz, 1966), etc.

Having segmented the macro pores in the void space, it is important to locate interconnects which are defined as the throat regions that separate macro pores. Note that two major pores may be connected by several isolated interconnects in foam scaffolds. In labelled macro pore segmentation, any voxel in a pore, which has neighbours in a different pore, is at an interconnect. Each connected group of interconnect voxels was labelled as an isolated interconnect in the network. Therefore all interconnects in the pore network can be located and labelled. Note that this definition gives a layer over 1 cell thick (i.e. it marks the voxels on the edges of both pores) and a thinning algorithm is required if a single voxel thick interconnect is required.
Figure 3.7 shows the segmentation of the pore network of a 70S30C bioactive glass sol-gel foam scaffold. The macro pores (Figure 3.7b) and interconnects (Figure 3.7c) were segmented from a cylindrical region of interest (ROI) (Figure 3.7a) of a micro-CT scan. The relative location of pores and interconnects were shown in Figure 3.7d. The interconnects in the scaffold were acting like windows to segment the pore space into isolated rooms (pores), which play an important role in terms of solute transport, cell migration, bone and blood vessel ingrowth \textit{in vitro} and \textit{in vivo}. Therefore, the interconnect sizes need to be accurately measured to evaluate the performance of bone scaffolds.

![Figure 3.7. 3D rendering of 70S30C bioactive glass foam segmentation. (a) cylindrical ROI of the scaffold; (b) segmented macro pores; (c) segmented interconnects; and (d) the segmented pores and interconnects placed in the scaffold (part). The diameter of the ROI was equivalent to 5 mm.](image)

3.4. Feature measurement

After segmentation, all the macro pores and interconnects were labelled and saved as separate volume files. The characteristics of interest need to be measured so that the scaffold can be quantitatively evaluated.

The pores in sol-gel bioactive glass foams are nearly spherical (as shown in Figure 3.8) since they are formed by bubbling; therefore, an appropriate quantitative measure is an equivalent
pore diameter for a sphere of the same volume as the pore. This is easily calculated from volume of labelled pores. The spatial resolution is normally finer than 10 µm when imaging bioactive glass foams. The macro pores are with equivalent diameters ranging from 300 µm to 900 µm. Therefore calculating the volume of the macro pores can be simplified by counting voxel amount, with a small error from partial volume effect.

![Figure 3.8. 3D rendering of a typical macro pore in bioactive glass scaffold: (a) isosurface and projection view of the pore; (b) the same pore compared with its equivalent sphere. The equivalent diameter of the pore is 605 µm.](image)

Although the interconnects between pores look like discs, they are randomly oriented relative to the orthogonal space in which the feature are scanned (as shown in Figure 3.7 c&d, and Figure 3.10a) and hence in prior studies they were quantified by the size of the bounding box around them. (Note, describing the interconnect size by volume is not appropriate as they are planar objects.) In this study, a new approach was developed, where the interconnects in bioactive glass foams are identified as planar discs of air separating spherical pores. Here, principal component analysis (PCA) was found to be useful to calculate the effective areas of interconnects in the bioactive glass foams. The algorithm is described as follows.

If an interconnect in a segmented image consists of $N$ connected voxels (Figure 3.10b). The $N$ voxels and their coordinates can be written in a matrix:

$$
\begin{bmatrix}
x_1 & y_1 & z_1 \\
\vdots & \vdots & \vdots \\
x_N & y_N & z_N
\end{bmatrix}
$$

Then the covariance matrix ($3 \times 3$) of the voxel coordinate matrix can be calculated and the eigenvalues and eigenvectors of the covariance were used to describe the principal orientation (axes in Figure 3.10b) of the interconnect. For disc-like interconnects, one of the eigenvalues
is much smaller than the other two. This eigenvector associated with the smallest eigenvalue is normal to the principal plane of the interconnect. The principal plane also passes the centroid of the voxels so it can be uniquely defined. The equivalent area of the interconnect was defined as area of the convex hull of all voxels projected to the principal plane (Figure 3.10c). Equivalent interconnect diameter was defined as the diameter of a circle with area equal to the effective area of the interconnect (Figure 3.10d).

In addition, the eigenvalues derived from PCA analysis can be used to form the equivalent ellipsoids of interconnects. An ellipsoid can be defined by:

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} + \frac{z^2}{c^2} = 1$$  \hspace{1cm} 3.3

For each interconnect, the square roots of the three eigenvalues are $a$, $b$, and $c$ respectively in Equation 3.3 to form its equivalent ellipsoid. Figure 3.11 a – c shows a typical interconnect in bioactive glass scaffolds and its equivalent ellipsoid, which qualitatively agreed well.

The principal component analysis can also be extended to quantify macro pores. After all the pores and interconnects were analysed with the PCA method, the original pore network can be simplified to a collection of ellipsoids. Another benefit of equivalent ellipsoids is they can be used to calculate the inscribed sphere/circle. In addition, this simplified network can be described analytically, allowing direct meshing for computer simulations, such as finite element analysis and computational fluid dynamic, or as an input for a network analysis of the structure (as shown in Figure 3.9) (Dong and Blunt, 2009).

Figure 3.9. Example of pore network topology in a 70S30C bioactive glass scaffold: (a) colour coded macro pores in the scaffold and (b) their connectivity.
Figure 3.10. PCA methodology for interconnect quantification: (a) typical interconnect in bioactive glass foam; (b) interconnect voxels rendered as spheres. The principal axes were calculated by the PCA method. Red, green and blue axes are the first, second and third principal axes respectively; (c) voxels rotated to the principal plane of the interconnect and the effective area was circled by 2D convex hull; (d) equivalent circle (solid circle filled with gray colour) and equivalent ellipsoid projected on the principal plane (dashed ellipse filled with green colour); (e) and (f) are equivalent circle and equivalent ellipsoid overlaid to original interconnect voxels respectively.
Figure 3.11. Equivalent ellipsoids for interconnects calculated by the PCA method. (a) Voxels belong to a typical interconnect in bioactive glass scaffolds; (b) the calculated equivalent ellipsoid of the interconnect; (c) overlapping of (a) and (b); (d) all the inner interconnects in a bioactive glass scaffold and (e) their equivalent ellipsoids.
3.5. Case study: melt-derived bioactive scaffold

A melt-derived bioactive glass foam scaffold was fabricated using the gel cast foaming method described in Wu et al. (Wu et al., 2011). The glass composition was ICIE16M: 49.46 mol.% SiO$_2$, 36.27 mol.% CaO, 6.6 mol.% Na$_2$O, 1.07 mol.% P$_2$O$_5$ and 6.6 mol.% K$_2$O. A typical glass foam, 6 mm in diameter and 8 mm high, was scanned using a commercial micro-CT unit (Phoenix X-ray Systems and Services GmbH, Wunstorf, Germany) at 80 kV and 50 µA, and with a voxel size of 9.06 µm.

The micro-CT image was processed to quantify the pore and interconnect size distributions of the foam. The image processing steps were as follows:

1. A 3×3×3 median filter was applied to remove noise in micro-CT scan (Figure 3.12c).
2. The image was then binarised using a global threshold. Isolated noisy struts were removed by a connected component labelling algorithm (Haralick and Shapiro, 1992). A representative slice was shown in Figure 3.12d.
3. A 5×5×5 morphological closing operation (dilation followed by erosion) was performed on the scaffold phase to remove the fine intra-strut porosity (Figure 3.12e), which otherwise affects the watershed transform.
4. A distance map was generated using the dilation based method described in Atwood et al. (Atwood et al., 2004).
5. A 3-D watershed algorithm (Mangan and Whitaker, 1999) was applied to the distance map, such that the pore space was segmented into macro pores. To increase the accuracy of the pore size distribution, the macro pores which were partially cut at the border of the ROI were eliminated from the segmentation (Figure 3.12f).
6. The interconnects were identified as isolated groups of voxels touching more than one macro pore.
7. After the pore space was segmented thoroughly, the pore size and interconnect size were measured using an equivalent sphere method and PCA based equivalent ellipsoid method respectively, as described earlier in chapter 3.4.
8. The pores and interconnect size distributions were plotted against both number density and also weighted by equivalent volume and effective area, respectively.
Figure 3.12. Micro-CT quantification of melt-derived ICIE16M bioactive glass foam. (a) 3D rendering of the sub image quantified and (b) 3D scaffold image with colour labelling according to voxel intensity; (c) a slice of the original scan after median filtering; a binarised 2D slice (d) before and (e) after morphological closing operation; and (f) the result of watershed segmentation. In (f), macro pores are colour labelled and blue colour indicates the pores which were partially cut at the border of the image, therefore not used for the size quantification.
Figure 3.12a is the 3D rendering of the sub image (i.e. ROI) of the glass foam. The structure of the scaffold was very porous and interconnected. The porosity of the sub volume obtained from micro-CT image was 60%. With micro-CT imaging, the heterogeneity of the struts density can be observed, as shown in Figure 3.12b and Figure 3.12c. Higher density regions (red colour in Figure 3.12b and brighter colour in Figure 3.12c) in the scaffold struts may refer to calcium rich phases.

![Graph](image)

Figure 3.13 Pore and interconnect size distributions for an ICIE16M scaffold using micro-CT: (a) pore size distribution, modal equivalent pore diameters are 271 µm and 425 µm, for number density and volume fraction distributions, respectively; (b) interconnect size distributions, with respective modal values of 115 and 128 µm for number density and area fraction distributions.

The pore and interconnect size distributions are shown in Figure 3.13. The modal equivalent pore diameter in number frequency distribution was 271 µm. The modal equivalent pore diameter by volume weighted method was much greater, with a modal value of 425 µm.
Although the volume weighted distribution was expected to have a larger mode than the number frequency distribution (Giddings et al., 1991), this 150 \( \mu \text{m} \) difference in modal values reflects that the smaller pores dominate the structure.

For the interconnects, the number density and weighted size distributions are very similar, with respective modes of 116 and 128 \( \mu \text{m} \), due to their being very sharp distributions centred just above 100 \( \mu \text{m} \). Note that both values are greater than 100 \( \mu \text{m} \) which is thought to be the minimum pore size for blood vessel ingrowth, suggesting that the melt-derived ICIE16M foam has a pore network suitable for a bone tissue engineering scaffold.

### 3.6. Summary

High resolution microtomography combined with 3D image processing has been shown to be a powerful tool for bone scaffold quantification. To quantify the 3D structure of glass foams, a new combination of different image processing algorithms was developed.

The quantification technique was demonstrated on the melt-derived ICIE16M bioactive glass foam and results showed that the scaffold was with a suitable pore network for bone tissue engineering applications. The modal equivalent diameters in volume/area weighted distributions were 425 \( \mu \text{m} \) and 128 \( \mu \text{m} \) for macro pores and interconnects respectively.

The new method presented in this chapter can accurately measure the orientation and size of each macro pore or interconnect in the pore network of a scaffold. Moreover, the micro-CT imaging process is non-invasive and non-destructive. Thereby, the structure properties of the same scaffold at different time points of a continuous experiment can be quantified and compared, such as in the dissolution study presented in Chapter 4.
4. **Quantifying the Dissolution of a 3D Bioactive Glass Scaffold in a Perfusion Flow Reactor²**

Bioactive glass has high potential for bone regeneration due to its ability to bond to bone and stimulate osteogenesis whilst dissolving in the body. Although 3D bioactive glass scaffolds with favourable pore networks can be made via the sol-gel process, compositional and structural evolutions in their porous structures during degradation *in vivo*, or *in vitro*, have not been quantified. In this study, bioactive glass scaffolds were put in a simulated body fluid flow environment in a perfusion bioreactor. Micro-CT was used to non-destructively image the scaffolds at different degradation stages. The new 3D imaging processing methodology described in Chapter 3 was used to quantify the scaffold’s pore size, interconnect size, and connectivity. The additional accuracy of using the principal component analysis based algorithm is demonstrated. During 28 days of dissolution, the modal interconnect size in the scaffold was reduced from 254 to 206 µm due to the deposition of mineral phases. However the pore size remained unchanged with a mode of 682 µm. The data presented is important for taking bioactive glasses scaffolds towards clinical products. It also demonstrates that the techniques described for imaging and quantifying scaffold pore structures as a function of degradation time may be applicable to many scaffold systems.

The non-destructive nature of micro-CT can enable the evaluation of samples *in situ*, where experiments such as compression tests and observation of mineral deposition can be performed as the scaffold is scanned (Ohgaki et al., 2006, Hagenmüller et al., 2007, Yue et al., 2010). In addition, the 3D scaffold images can be used to generate meshes for computational

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² Note, this chapter is based on the following paper arising from my thesis: Yue, S., Lee, P. D., Poologasundarampillai, G. and Jones, J. R. (2011), 'Evaluation of 3D bioactive glass scaffolds dissolution in a perfusion flow system with X-ray microtomography', Acta Biomaterialia, 7, 2637-2643
Fluid dynamic and mechanical simulations (Porter et al., 2005, Jones et al., 2007c, Singh et al., 2009). Therefore, many aspects of the performance of scaffolds can be evaluated and then improved in a non-invasive and quantifiable manner, reducing product design costs.

Current bioactivity and degradation tests for bioceramics usually consist of immersing a sample in a fixed volume of simulated body fluid (SBF) and placing it in an incubator and then observing the final extent of HCA formation. However, bioactive glasses, and many other bioceramics, are dynamic materials which undergo dissolution before a HCA layer can deposit on their surface. A test that quantifies this precursor stage is required. For example, a high glass concentration can cause calcite to deposit on the glass surface instead of HCA (Jones et al., 2001). The in vivo environment is not a closed environment and saturation of ions is not expected. Therefore, the pre-screening process for new scaffolds needs improvement. Perfusion bioreactors are widely used in vitro in tissue engineering applications because perfusion flow can enhance mass transport, introduce shear stress, and thereby improve tissue growth (Porter et al., 2007). The aim of this work is to use a perfusion bioreactor with a local inhomogeneous flow rate, combined with micro-CT imaging and image analysis, to study the degradation behaviour of bioactive glass foam scaffolds in a simulated body fluid (SBF) flow environment. The hypothesis is that the porous scaffold would undergo dissolution over time, causing the interconnects of the pores to open. Secondary objectives were to determine whether the deposition of the HCA layer could be observed using this technique; whether changes in pore size could be measured and whether preferential flow within the scaffold would cause heterogeneous dissolution.

4.1. Materials and methods

4.1.1. Bioactive glass scaffolds synthesis

Bioactive glass foams of 70S30C composition (70 mol.% SiO₂, 30 mol.% CaO) were prepared using a sol-gel foaming method as previously described (Sepulveda et al., 2002a, Jones et al., 2006a). The sol preparation began with mixing 0.2 N nitric acid with deionised water using a magnetic stirrer, followed by addition of tetraethyl orthosilicate (TEOS) and calcium nitrate (all Sigma-Aldrich) in order. The initial molar ratio of water to TEOS (R ratio) was 12:1. Aliquots of 50 ml of the sol were combined with 3 ml of 5 vol.% hydrofluoric acid (HF, catalyst) and 0.35 ml of Teepol (surfactant, Thames Mead Ltd, London) and then foamed using vigorous agitation. As the foamed sol approached the gelation point, it was cast
into cylindrical Teflon® moulds, sealed and aged at 60 °C for 72 h. Previous work has shown Teflon® moulds are non-reacting and they improve the homogeneity of the glass composition (Lin et al., 2010). The samples were dried in air for a total of 94 h at 60 °C, 90 °C and 130 °C. Finally, the scaffolds were stabilised at 600 °C for 3 h and then sintered at 800 °C for 2 h. The thermal processing was optimised in previous studies, with the drying process designed to prevent cracking and the sintering used to improve compressive strength (Jones et al., 2006a).

### 4.1.2. Perfusion bioreactor system

A simulated body fluid (SBF) solution, with ion concentrations (Corrected SBF in Table 2-2) similar to those of human extracellular fluid, was prepared following the Kokubo method (Kokubo, 1991, Kokubo and Takadama, 2006). SBF was chosen, rather than cell culture medium or other buffer solution, to allow comparisons with previous study (Jones et al., 2006a). Cylindrical bioactive glass scaffolds (weight 0.13 ± 0.2 g) 6 mm in diameter and 7 mm high, together with a length of non-degradable silica glass fibre, were wrapped with Teflon tape and inserted into a Teflon® ring (termed a scaffold assembly). (Note that the silica glass fibre was added to calibrate the X-ray attenuation in the micro-CT, enabling the change in density of the scaffold to be monitored.) The Teflon® ring containing the sample was then placed into the cylindrical chamber of a perfusion bioreactor (Gradient container, Minucells Minutissue, Weinheim, Germany (Seitz et al., 2007)), as illustrated in Figure 4.1. In order to prevent flow from bypassing the scaffold, the outer diameter (12 mm) of the Teflon® ring matches the inner diameter of the chamber, while the Teflon® ring was also wrapped with Teflon® tape to ensure a tight seal. Three samples were run in separate experiments on separate occasions using fresh SBF, using the same perfusion chamber.

The SBF flow circulation, which was directed through the scaffold from bottom to top with flow rate of 1 ml/min, was maintained with a peristaltic pump (Masterflex, model 07519-25, Cole-Palmer Instrument Co., Illinois, USA). Note a relative high flow rate was used to accelerate the impact of perfusion flow on the dissolution behaviour of the foam scaffold. A 1 L reservoir of SBF was maintained at 37 °C in an incubator. The experiment was repeated for 3 bioactive glass scaffolds of the same porosity to obtain values of uncertainty.
Figure 4.1. Schematic of the dissolution experimental setup. Arrows on silicone tubing indicates SBF flow direction.

4.1.3. Optical emission inductive coupled plasma spectroscopy

For each of the three samples, at 0 h, 1 h, 8 h, 24 h, 7 d and 28 d of the dissolution time point, 50 ml of the SBF solution was collected for analysis. The entire SBF of 1 litre was replaced after 7 d to reduce the chance of bacterial contamination. The concentrations of P, Si and Ca in the collected solutions were measured with optical emission inductive coupled plasma spectroscopy (Thermo Scientific ICP Spectrometer, Model iCAP 6300 Series Duo).

4.1.4. X-ray microtomography

X-ray microtomography (micro-CT) was used to scan the scaffold assembly as a function of degradation time in SBF flow. Each scaffold assembly was scanned before the dissolution study using a lab-based micro-CT unit (Phoenix X-ray Systems and Services GmbH, Wunstorf, Germany) at 100 kV and 100 µA, and with a voxel size of 8 µm. At 24 h, 7 d and 28 d, the dissolution experiment was paused and the scaffold assembly was removed out from the bioreactor, dried at 37 °C overnight and re-scanned by micro-CT with same setting as that of the 0 h scan. After micro-CT scanning, the assembly was re-installed and the dissolution experiment resumed.
**4.1.5. Scanning electron microscopy and energy dispersive X-ray spectrometry**

Two additional bioactive glass scaffolds were used for electron microscopic analysis as it is destructive – one was imaged unreacted, one immersed in SBF flow for 3 d and then imaged, and one of the 3 repeats was also imaged after 28 d in SBF. Each was dried and imaged using a scanning electron microscope (SEM, Leo 1525) with energy dispersive X-ray spectrometry (EDX) to characterise morphology and composition. For the 28 d sample, a micro-CT scan was performed prior to SEM and EDX analysis to provide confirmation of the micro-CT data interpretation.

**4.1.6. Three-dimensional image analysis**

The 3D image analysis methodology was as described in Chapter 3, with the following additional steps to register the images to each other spatially, and to normalise the intensity levels:

1. A 3×3×3 median filter was applied to all micro-CT images to remove noise.
2. For each scaffold assembly, the micro-CT images at 24 h, 7 d, and 28 d, were numerically registered to the appropriate image taken at 0 h. The registration process re-sampled the images first (by a initial factor of 4 and a refined factor of 2), and then transformed the moving image rigidly (Rigid3D transform, only rotation and translation), based on the Mean Squares similarity metrics using ITK (Ibáñez et al., 2005) (Insight Segmentation and Registration Toolkit, ITK).
3. The Teflon® ring and non-degradable silica glass fibre in the micro-CT images were thresholded and then located using a connected component labelling algorithm (Haralick and Shapiro, 1992). The X-ray attenuation properties of the Teflon® and silica glass fibre were assumed to remain constant, allowing all the micro-CT images to be normalised to each other.
4. Global thresholding was applied to the normalised images to classify each voxel as either scaffold or background according to its intensity. The thresholding values were determined from histograms of the images, picking the value equidistant for the peak background and scaffold levels.
The PCA methodology described in Chapter 3 was then applied, segmenting and labelling the structures as macro pores and interconnects, differing significantly from previous studies (Atwood et al., 2004, Jones et al., 2007c). Figure 4.2 shows each stage of the process. The dissolution study is the second example of application of the new method and differs slightly to the method used to quantify the scaffolds in Chapter 3. The new methodology first located the principal plane of each interconnect (circular disk shape in Figure 4.2c) by a principal component analysis (PCA) method. The steps used to find the principal plane and then measure the interconnect size are:

5. For each interconnect, the PCA began with calculating the covariance matrix of its voxels coordinates.

6. The eigenvector which corresponded to the smallest eigenvalue of the covariance matrix is the normal to the interconnect’s principal plane. The principal plane also passes the centroid of the interconnect voxels. Therefore, for each interconnect in the 3D space, there is only one unique principal plane and which can be located by its normal and the centroid of the interconnect.

7. In order to measure the interconnect size, all voxels of a given interconnect were projected to its principal plane. This allows the 3D disk-like shape to be reduced to a 2D projection. A convex hull algorithm was then used to determine the effective area of the interconnect’s projection in 2D. An equivalent diameter was also determined equal to the diameter of a circle with equal area to the interconnect.

8. The distributions of the pore size and interconnect size for each scaffold were then produced, weighted by volume fraction or equivalent area fraction, respectively.

This new method of performing a principal component analysis of the interconnects and then measuring them in the principal plane significantly improved the accuracy of the dimensions obtained, as shown by comparing Figure 4.2e & Figure 4.2f. Figure 4.2e & Figure 4.2f are showing the same interconnect of a scaffold, but Figure 4.2e shows the original bounding box method used to quantify its equivalent diameter, whereas Figure 4.2f shows the accuracy of the PCA method. The bounding box diagonal of the single interconnect was 414 µm (using the method from (Atwood et al., 2004)), whereas the equivalent diameter of the same interconnect obtained by the PCA method was 274 µm, showing a 51 % improvement over the old method.
Figure 4.2. 3D rendering of micro-CT images show: (a) a 3D porous bioactive glass foam scaffold; (b) a sub volume of the scaffold containing two colour labelled pores (scaffold struts rendered with 50 % transparency) identified by the dilatation and watershed algorithms; (c) the two pores from (b) with their neighbouring interconnects; and (d) equivalent pores and interconnects of (c) from the quantification algorithms; (e) a single interconnect with its coordinate bounding box. The bounding box diagonal which was used as equivalent diameter by Atwood et al. (Atwood et al., 2004) was 414 µm in length; (f) the same interconnect in (e) which was rotated to its principal plane. The length of the equivalent diameter obtained by PCA method was 274 µm. In (c) and (d), pores were rendered with 50 % transparency, and each cone indicates the normal of the principal plane for every interconnect.
4.2. Results

High resolution X-ray microtomography (micro-CT) allowed the non-destructive scanning of the same bioactive glass scaffold at a series of dissolution time points, and through registering these scans to each other, a highly accurate quantification of the dissolution kinetics was determined. The changes in one scaffold at four dissolution time points is shown in Figure 4.3a-d as a 3D rendering and as 2D slices in Figure 4.3e-h (perpendicular to the flow direction). The regions with higher intensity value in the volume image indicate where the material has a higher X-ray attenuation, and was rendered with warmer colour in Figure 4.3. The scaffold phase had a higher intensity level than the background phase which was easily distinguished. The amount of attenuation was a combination of both the composition (higher atomic number atoms attenuate more) and density – correlations between the micro-CT scan and SEM-EDX illustrated that the red regions contained a greater percentage of calcium. Figure 4.3a & e showed the initial pore network prior to dissolution (0 h). The colour variation of the struts reflected the inhomogeneous initial composition within the amorphous scaffold; note that the high X-ray attenuating material was observed more frequently close to the surface of the struts.

After 24 h in SBF flow, there was a slight reduction in overall image intensity level (comparing Figure 4.3b & f to Figure 4.3a & e). However, the reduction was quite heterogeneous: the voxel intensity reduced much more in some regions than others, whilst in some regions the intensity increased. These observations suggest that in SBF flow conditions, material leached out from the struts of the scaffold unevenly, while redepositing at other locations.

After 7 d immersion (Figure 4.3c & g), the voxel intensity level of the volume image significantly reduced compared to the 0 h and 24 h images, indicating dissolution of calcium. In addition, there was a significant decrease in the size of many interconnects, with only a few increasing in size. After 7 d there was also a number of regions of localised deposition of highly attenuating species at the periphery of a number of pores in the top left-hand corner of the scaffold (red and yellow regions), which were not present at 0 h and 24 h. As confirmed below using EDX, these were depositions of Ca rich mineral phases. After 28 d immersion (Figure 4.3d & f), almost all areas of the scaffold showed reduced attenuation. The exceptions were the upper left region in 3D image, and top and bottom regions in the 2D slice,
which had highly attenuating (red) regions in the walls, and around the periphery of many of the pores. The deposition of Ca rich phases that began at 7 d seems to have increased at 28 d, shown by an increase in red intensity at the pore surface in Figure 4.3d compared to Figure 4.3c.

Figure 4.3. 3D sub volumes and transverse slices (perpendicular to the flow direction) from the normalised μCT images of the scaffold at time points of: 0 h (a, e); 24 h (b, f); 7 d (c, g); and 28 d (d, h). (Note, the colours indicate relative X-ray attenuation and are all normalised to each other. Warmer colour indicates higher attenuation material.)
Figure 4.4. Size distributions of the equivalent diameter of the: (a) pores; and (b) interconnects.

The quantification illustrates that all pores were well connected with a mean of 5 disc-like interconnects to neighbouring pores, 3.6 (72%) of which, on average, had an equivalent diameter greater than 100 µm (Figure 4.4b). The modal equivalent pore diameter remained unchanged at 682 µm over the four time points (Figure 4.4a); however, there was a slight broadening of the pore size distribution at 28 d as deposition on the insides of the pores occurred. Prior to dissolution, 95 % (in area) of interconnects had an equivalent diameter larger than 100 µm, with modal value of 254 µm. After 24 h, the distribution became skewed to slightly smaller interconnect sizes (modal value of 254 µm), reducing further to 206 µm after 7 d. The reduction in modal interconnect size halted after 7 d, remaining at 206 µm at 28 d. Note that although the mean modal diameter reduced, there were locations where the interconnect size enlarged, but this was less common. Throughout the dissolution process 95 % of the interconnects remained larger than 100 µm, providing an easy transport mechanism of nutrients and cells between macro pores.
The struts in the unreacted foams had a uniform surface (Figure 4.5a) and the EDX analysis (Figure 4.5d) gave a composition similar to the nominal composition of the bioactive glass, i.e. 70 mol.% of SiO₂ and 30 mol.% of CaO. After 3 d in SBF flow the scaffold's surface roughened in some regions (Figure 4.5b), perhaps due to partial dissolution. EDX (Figure 4.5e) in these regions showed a significant reduction in Ca, supporting the calcium dissolution hypothesis. After 28 d (Figure 4.5c), a material with a crystalline appearance covered most scaffold surface areas. EDX analysis (Figure 4.5f) of this deposit indicated high calcium and phosphorous levels, suggesting that an HCA layer formed. The presence of this crystalline structure was observed on all the scaffold surfaces observed in the SEM for the 28 d sample.

Figure 4.5. SEM images (a – c) and associated EDX (d – f) analysis of the 70S30C bioactive scaffolds at 0 h (a, d), 3 d (b, e), and 28 d (c, f) time points.
Changes in the SBF Si, P, and Ca levels with time relative to the initial composition were plotted in Figure 4.6, as determined by ICP analysis. During the first 7 d, the Si level increased by 8.8 ppm (Figure 4.6a). The P levels decreased after 7 days, probably due to phosphate depositing on the glass surface as calcium phosphate rich phases (Figure 4.6b) [26]. Ca levels increased slightly in the first 24 h (Figure 4.6c), due to an initial burst of Ca dissolution, exceeding the rate of redeposition. After 7 d, the Ca level decreased to 5.7 ppm lower than fresh SBF after 7 d, indicating the deposition of Ca rich phases dominates over dissolution. After sampling at 7 d, the SBF solution was completely replaced with fresh medium. Over the next 21 d, the Ca level and P level dropped considerably, indicating further deposition of calcium phosphate rich phases (as confirmed via EDX above and the decrease in pore size in Figure 4.4a).

Figure 4.6. ICP analysis for the: (a) Si, (b) P, and (c) Ca ion concentrations in SBF solution at each time point.
4.3. Discussion

The non-destructive and non-invasive nature of micro-CT enabled the capture of the changing structure of bioactive glass scaffolds during dissolution for the first time. Quantification of the micro-CT scans of the sol-gel derived 70S30C bioactive glass foam showed that throughout 28 d of degradation it retained its interconnected porous network suitable for bone regeneration applications both in terms of pore size and interconnect diameter. The pore size distribution was with a mode of 682 µm, which did not change over 28 d of immersion. The macropores within the network were well connected (with an average of 3.6 large interconnects per pore). More importantly, the majority of interconnects (95% of their total area) had equivalent diameters in excess of 100 µm, which meant they were potentially large enough for vascularised bone growth throughout the scaffold.

When characterising the porous structure of a scaffold, a transition from qualitative interpretation to quantitative measurement was made possible with the novel 3D image analysis techniques. The 3D scan of the scaffold was segmented using a sequence of image processing algorithms as described in the Methods section above. The measurement of the interconnect sizes has been made more accurate compared to previous work (51% for the example given in the Methods Section), and fully automated by introducing a principal component analysis (PCA) based algorithm. The PCA algorithm allowed objects that were randomly oriented in 3D space to be aligned such that an optimal projection onto a 2D plane is achieved and a more accurate measurement was obtained (a 50% improvement in the example given). Although this method was demonstrated using interconnects in this study, the methodology is applicable to a wide range of features, from bone nodules to fibrous features in composite scaffolds. This improvement obtained by this method is limited to objects which are elongated in either one or two directions (i.e. that have one or two strong principal components).

The micro-CT quantification showed that calcium distribution in the unreacted scaffolds was not homogenous. The sol-gel process was previously thought to be a method of choice for producing homogeneous compositions. However, previous work has shown that the use of calcium nitrate as a calcium precursor, which is the traditional choice, does not give a homogeneous calcium distribution (Lin et al., 2010). Here, the micro-CT showed this to be true in foams. The inhomogeneity was due to the calcium nitrate being soluble in water and
calcium not going into the silica network until a temperature of 400 °C was reached. After drying, the struts consist of silica nanoparticles coated with calcium nitrate. During stabilisation and sintering the nanoparticles fuse together and the calcium nitrate dissociated and calcium diffused into the struts. The foams have greater homogeneity than monoliths but cannot be considered completely homogeneous.

During dissolution there were two dominant events: dissolution of the scaffold followed by mineral deposition. Over the first 24 h, calcium content was depleted near the surface of the scaffold due to ion exchange of calcium ions with H+ from the solution. Soluble silica was also lost to the solution. The micro-CT quantification showed that the majority of interconnects shrank slightly during the first 7 d, but the pore size remained similar. The interconnect diameter reduced as calcium phosphate deposited on the surface of the scaffolds, growing into the interconnect region. This was confirmed both by ICP, where a reduction of Ca and P was measured in SBF, and via SEM-EDX, where deposits were visually observed and Ca and P quantified by EDX. In addition micro-CT showed a decrease in calcium (shown by X-ray attenuation) within the scaffold and an increase in dense/high atomic number deposits on the pore surfaces, including near the interconnects. Note that a few interconnects enlarged, indicating preferential flow channels formed in the perfusion bioreactor causing dissolution of the glass at edges of these interconnects. Unfortunately since a laboratory source micro-CT machine was used with polychromatic X-ray, only the relative changes in calcium level, and not the absolute levels, could be determined.

The 3D micro-CT images and image analysis indicated that there was little change in the pore morphology between 7 and 28 days, which implies that the silica was not continuing to degrade. ICP results agree with this, as silica content of the SBF increased less between 7 and 10 days compared to 0-7 days. This is quite likely in flow conditions as local pH must be greater than 9 for Si-O-Si bonds to break and for soluble silica to be released. A local pH of greater than 9 is quite likely in the first 7 days as the calcium was released by the glass. The flow then removed the calcium ions from close to the glass, causing a buffering effect. The HCA deposition will also reduce the surface area of the glass that is in contact with the SBF. Bioactive glasses have been found to degrade in vivo, therefore long term degradation may be due to the action of osteoclasts. This is preferable to continual degradation by aqueous dissolution as degradation by continuous dissolution may be too fast to allow bone remodelling to occur, which may take weeks to months depending on the defect site.
4.4. Summary

High resolution X-ray microtomography was used to non-destructively image 3D sol-gel derived bioactive glass scaffolds during the dissolution process in SBF flow for the first time. The 3D image analysis techniques required significant improvement from previous work to provide accurate quantification. This was done by introducing a principal component analysis (PCA) based method to fully automatically measure the interconnect sizes within the scaffold, providing an increased accuracy. The quantification shows that the sol-gel derived 70S30C bioactive glass foam has a suitable porous structure for use as a bone tissue engineering scaffold. The pore size remained unchanged with a mode of 682 µm, whereas the interconnects reduced slightly in size (254 to 206 µm) due to the deposition of mineral phases. A crystalline phase matching the composition of hydroxycarbonate apatite (HCA) was found to form over the majority of the scaffold after 28 days in a SBF flow environment.
5. **Quantification of Selective Laser Melted Titanium Foams for Orthopaedic Applications: Methods**

5.1. **Introduction**

In this Chapter, methods for assessing the accuracy and efficacy of selective laser melting (SLM) for the production of Ti scaffolds are explored, with a particular focus on orthopaedic fixation devices with controlled porosity and structure randomisation (Mullen et al., 2009, Mullen et al., 2010). Such methods are required in order to compare the final product with the original design and to quantify structural parameters, such as pore size distribution and the 3D surface roughness profiles of constituent struts.

Traditional dimensional measurement techniques, such as coordinate-measuring machines (CMM), scanning electron microscopy (SEM), or interferometry, cannot assess the complex internal features of these implants. Therefore, micro-CT was selected as the most suitable process to non-destructively image the internal structure of the samples in 3D. Building on the image processing methodologies developed in Chapters 3 and 4, further extensions were developed to quantify these more complex 3D structures, thereby producing a powerful tool for the structural characterisation of these porous implants. These extended tools are still applicable to the bioactive foams; it is hoped they will be applied to many other orthopaedic implants in the future, since the techniques can be shown to be independent of implant material, structure, and scale.
5.2. **Scaffold design and geometric properties**

5.2.1. **Sample design and preparation**

All samples for this study were produced by SLM using commercially available pure titanium powder (Sumitomo, Japan) using an MCP Realizer 2, 250 SLM system (MTT, UK) and were provided by the University of Liverpool. A schematic of the SLM machine and its associated optical system for positional control of the laser beam is shown in Figure 5.1. The full manufacturing details have been given previously (Mullen et al., 2009), therefore only an overview is provided here.

![Schematic of the SLM process](image)

**Figure 5.1.** Schematic of the SLM process. Modified from Mullen *et al*. 2009 (Mullen *et al*., 2009).

The process uses a Ti powder. SEM images of the highly spherical, gas atomised powder are shown in Figure 5.2a. The powder had a relatively wide log-normal particle size distribution with a modal particle diameter of 28.5 µm, with 90% of the particles being smaller than 49.8 µm in diameter (Figure 5.2b).

The porous structure of the scaffolds was designed using the Manipulator software package (version 4.7) developed by the University of Liverpool and based on a unit cell approach (Mullen *et al*., 2009). To produce the design, a bounding box larger than the overall geometry of the scaffold is formed, and then sub-divided into individual mesh cells (see Figure 5.3a). The unit cell structure is then replicated within this mesh (Figure 5.3b). The cubic unit of the mesh grid used in this study, i.e. the unit cell, had an edge length of 600 µm. Next, each unit
cell within the bounding geometry was replaced with an octahedral topology unit to form the octahedral wire frame network illustrated in Figure 5.3c and Figure 5.4a.

![Figure 5.2. Typical feed-stock Ti powder: (a) SEM images (b) and particle size distribution (Courtesy of Dan Jones, University of Liverpool).](image)

In Figure 5.4a, two connected unit cells are displayed as blue cubes and the octahedral network is shown using gray wire frame. An octahedron with an aspect ratio of 1:1:1 was formed by two square-based pyramids concatenated from two neighbouring unit cells. As shown in Figure 5.4b – d, pseudo-randomness can be introduced into the geometries by perturbation of the Cartesian coordinates of the grid vertices (displayed as red spheres in Figure 5.4) with respect to the regular design by 10 %, 20 %, and 30 % (in this case along the x, y, and z axes) of the unit cell size. The perturbation technique also ensures that strut integrity is maintained at all nodes (Mullen et al., 2010).

To export the wire frame network design for SLM manufacturing, it is virtually sliced at a vertical interval of 50 µm (i.e. the slicing interval, $l_{\text{slicing}}$), and the coordinates of intersections are exported to a *f&s file which is in a format suitable for upload to the SLM
machine (Fockele and Schwarze, 1994, Jamieson and Hacker, 1995). These exported coordinates are correlated to the discrete laser melting centres during manufacturing.

Figure 5.3. Schematic of the porous design by a unit cell approach. (a) a bounding cylinder; (b) the mesh grid domain; (c) cubic grid cell within the bounding volume filled by the octahedral wire frame unit; (d) final cylindrical scaffold design with octahedral unit cells.

Figure 5.4. Octahedral network with (a) 0 randomness; (b) 10 % randomness; (c) 20 % randomness; and (d) 30 % randomness. The unit cell is illustrated as a blue cube with vertices shown as red spheres. The gray wire frame indicates the topology of the porous structure.
The strut diameter ($D_{\text{strut}}$) of the SLM built scaffold is governed by laser energy, laser spot size, and powder property. When manufacturing with identical conditions, the diameter of the strut is also affected by its local building angle to the powder bed (Mullen et al., 2009).

![Strut diameter measured from SEM image of UC600 regular scaffold (Courtesy of Dan Jones, University of Liverpool).](image)

To test the characterisation techniques simple cylindrical scaffolds (4 mm in diameter and 4 mm high) with an octahedral unit cell size of 600 µm (termed UC600), and structural randomness of 0, 10 %, 20 %, 30 %, were fabricated and micro-CT scanned. Additional scaffolds were also fabricated and the strut diameter measured to be 180 µm using SEM (Figure 5.5); this value was assumed to equal to the designed strut diameter ($D_{\text{strut}}$) and diameter of laser melted sphere ($D_{\text{melting}}$) for topological network analysis and scaffold quantification.

### 5.2.2. Geometric analysis of scaffold designs

The geometric and topological properties of the octahedral wire frame networks were analysed from the laser melting positions in the associated f&s files. The connectivity among discrete laser melting positions (termed nodes) was assumed to be governed solely by spatial distance. For regular design, the wire frame network has repeated units and symmetrical geometry. The geometric properties of the UC600 regular scaffold are summarised in Table 5-1 and illustrated in Figure 5.6.
### Table 5-1. Geometric and topological properties of the regular UC600 scaffold

<table>
<thead>
<tr>
<th>Name</th>
<th>Notation</th>
<th>Formula</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit cell size</td>
<td>$l_{uc}$</td>
<td>$Design parameter$</td>
<td>600 µm</td>
</tr>
<tr>
<td>Octahedron edge length</td>
<td>$l_{edge}$</td>
<td>$l_{edge} = \frac{\sqrt{3}}{2} l_{uc}$</td>
<td>520 µm</td>
</tr>
<tr>
<td>Octahedron edge azimuth angle</td>
<td>$\theta$</td>
<td>$\theta = \sin^{-1} \frac{l_{uc}}{2 \times l_{edge}} = \sin^{-1} \frac{\sqrt{3}}{3}$</td>
<td>35.3°</td>
</tr>
<tr>
<td>Major pore diameter</td>
<td>$D_{major}$</td>
<td>$D_{major} = l_{edge} - D_{strut}$</td>
<td>340 µm</td>
</tr>
<tr>
<td>Interconnecting cylinder diameter</td>
<td>$D_{cylinder}$</td>
<td>$D_{cylinder} = \frac{\sqrt{2}}{2} l_{uc} - D_{strut}$</td>
<td>244 µm</td>
</tr>
<tr>
<td>Melted sphere diameter</td>
<td>$D_{melting}$</td>
<td>$SLM parameter$</td>
<td>180 µm</td>
</tr>
<tr>
<td>Strut diameter</td>
<td>$D_{strut}$</td>
<td>$Design parameter$</td>
<td>180 µm</td>
</tr>
<tr>
<td>Slicing interval</td>
<td>$l_{slicing}$</td>
<td>$Design parameter$</td>
<td>50 µm</td>
</tr>
</tbody>
</table>

**Figure 5.6.** Geometric and topological properties of the UC600 regular design: (a) two octahedral unit cell; (b) laser spots (nodes) from an f&s file; nodes connectivity for (c) intended design and (d) effective topology.
Figure 5.6c shows the intended design of the UC600 regular octahedral wire frame network. Nodes (shown as balls) were connected into struts (and shown as sticks) at a spacing of 87 µm for this structure. However, as the effective laser melting sphere diameter was 180 µm, nodes from different octahedron edges were also connected when close to the octahedron vertices. Therefore the effective topology differs from the intended design, as shown in Figure 5.6d, where the nodes close to the octahedron vertices are clustered and different from the original octahedral topology.

When pseudo randomness is introduced into the scaffold design, the intended topology is further perturbed as several octahedron vertices can merge into one big cluster. The topology may also affected by the virtual slicing process. Given the slicing interval \( l_{\text{slicing}} \) is 50 µm, the critical azimuth angle (or building angle) is 16.1°. This means nodes which belong to an edge with an azimuth angle \( \theta \) less than 16.1° will be disconnected from each other. This is illustrated in Figure 5.7.

![Figure 5.7. Schematic of the node connectivity of an edge with different azimuth angles in (a) UC600 regular design; and (b) disconnected nodes in a random design.](image)

The effective topologies of UC600 scaffold designs with graded randomness are shown in Figure 5.8. In the 10% randomised design (Figure 5.8b), the octahedral network remains clear. In more randomised designs (Figure 5.8c & d), however, the topologies cannot be viewed as octahedral networks.
Figure 5.8. Topology network of scaffold designs with (a) 0% randomness; (b) 10% randomness; (c) 20% randomness; and (d) 30% randomness. Two nodes are connected when the distance between them is less or equal to 180 µm. Warmer colours indicate higher degrees of connectivity. In (d), the dashed box highlight four nodes which belong to the same octahedron edge, but are disconnected with each other.

The theoretical pore size definition in the regular design based on that defined in Mullen et al. 2009 (Mullen et al., 2009). The major pore (Figure 5.9a – b) is defined as the inscribed sphere in an octahedron unit. The interconnecting cylinder (Figure 5.9c – d) is defined as the inscribed cylinder which can be inserted into the scaffold from three orthogonal directions.

Both characteristics are governed by unit cell size and strut diameter. The diameters of the major pores ($D_{\text{major}}$) and interconnecting cylinder ($D_{\text{cylinder}}$) for a UC600 regular scaffold with strut diameter of 180 µm were 340 and 240 µm respectively (Table 5-1).
Because the octahedral wire frame network is symmetrical, three major pore segmentations can be found with respect to the different orthogonal axes, as shown in Figure 5.10a – c. Figure 5.10d shows the relative spatial relationships between these segmentations, which are competing for void space (i.e. neighbouring major pores with respect to different axes merge into each other).

Figure 5.10. Axis dependent major pore segmentations with respect to (a) $x – axis$; (b) $y – axis$; (c) $z – axis$; and (d) all three sets of segmentations.
5.3. **Micro-CT quantification of the UC600 scaffolds**

In this study, micro-CT coupled with 3D image processing algorithms was used to characterise the SLM built scaffolds.

The input files of the SLM system, f&s files, were analysed and used to provide prior knowledge of the pore network. Ideal volume images were also generated based on the f&s files of scaffold designs in order to directly compare them to the micro-CT reconstructions of the real productions. Algorithms for quantifying scaffold properties, such as pore size distributions and 3D surface roughness, were developed and applied to micro-CT images.

### 5.3.1. **Micro-CT imaging and pre-processing**

Both the UC600 regular and 30% randomised scaffolds were micro-CT scanned at a voxel size of 9.06 µm using a lab-based facility (Phoenix X-ray Systems and Services GmbH, Wunstorf, Germany) with an accelerating voltage of 100 kV and a filament current of 100 µA. The UC600 regular scaffold was also scanned at a voxel size of 4.5 µm for surface roughness quantification.

For each micro-CT image, a $3 \times 3 \times 3$ median filter was applied to remove noise and then the filtered image was binarised into scaffold phase and background phase by global thresholding (as previously described in section 3.2.3). The threshold value was chosen to be equidistant between the Ti phase peak and void phase peak in the histogram of the image.

After binarisation, the open porosity and closed (isolated) porosity in the scaffold can be identified by a connected component labelling algorithm and quantified by voxel number counting.

### 5.3.2. **Voxelisation and registration**

The voxelisation process, which turns computer models into 3D volume images, enabled direct comparison between ideal designs and real productions. The SLM building process can be simulated from the laser melting positions (nodes) provided by the f&s file to create a representative volume image. As such, the voxelised image represents the ideal geometry without dimensional or manufacturing error.
For each scaffold design, a blank volume was created to represent the regularly gridded bounding box (slightly oversized) of the scaffold. In the created volume, the voxels indicating the discrete nodes were set up as foreground voxels. The selective laser melting process was then mimicked by convolving a melting kernel to the created volume.

For a spherical convolving kernel, the convolution processing can be simplified to a distance-transform based isotropic dilation operation, as the kernel is symmetrical in 3D. The detailed algorithm used to construct the representative volume image is described below:

1. The coordinates list of the nodes was read from the f&s file of the scaffold design. The size of the bounding box is the dimensional difference of the coordinates added to the diameter of the spherical melting kernel.
2. An empty volume was created to represent the bounding box of the scaffold. Choosing the voxel size of the created volume is a trade-off between image size and voxelisation accuracy. The voxel size for the micro-CT scans was 9 µm and the computer designs were vertically sliced at 50 µm interval when exported as f&s files, therefore voxel size of the created volume was set to 5 µm. The nodal coordinates could then be converted to the relative voxel coordinates with respect to the origin of the bounding volume.
3. In the created volume, the voxels indicating the nodes were set up as foreground voxels and a Euclidean distance transform was then applied to produce a distance map. In this 3D distance map, the scaffold phase was separated according to the diameter of the spherical melting kernel. To form a scaffold with strut diameter of 180 µm, any voxel with a distance value less or equal to 18 (which represent a spherical kernel of 90 µm radius, given a voxel size of 5 µm) was selected to represent the scaffold phase. The remaining voxels were assigned to the background. Note that the strut diameter of 180 µm was estimated from previous SEM images.

The node topology information of the scaffold design can also be integrated into the voxelisation process. For example, in Figure 5.11a, the nodes of a UC600 regular design were classified as struts nodes (shown in blue) or vertices nodes (shown in red) depending on their degree of connectivity. These two types of nodes can be used to create separate volumes which will then be merged together so that their associated voxels are labelled with different integers (Figure 5.11b). When comparing the strut roughness of the real production to the
design, the position and local orientation of each isolated strut segment (Figure 5.11c) can be calculated from this labelled-volume.

![Figure 5.11](image)

Figure 5.11. Voxelisation of a UC600 regular scaffold from an f&s file. (a) vertex nodes (red) and struts nodes (blue) in the design; (b) voxelised image with labelled vertices and struts voxel groups; and (c) labelled isolated strut segments.

After voxelisation, numerical registration of micro-CT scans of scaffolds to their associated design volume images was achieved using an open source image processing library ITK (Insight Segmentation and Registration Toolkit). The registration process re-sampled the images by an initial factor of 4 and a refined factor of 2, refining the transform based on the mean squares similarity metric (Ibáñez et al., 2005). Once the transform was achieved, the micro-CT scanned volume was aligned with the design volume using the ITK Rigid3D transform (which applies only global rotation and translation).

After registration, the percentage of volume difference of the design volume and the micro-CT scan of the SLM built scaffold can be compared and quantified.

### 5.3.3. Regular structure segmentation

As shown in Figure 5.10d, major pores with respect to different orthogonal axes are competing for void spaces in the pore network of the regular design. Therefore a unique segmentation, called interconnecting pore segmentation, can be achieved using micro-CT volumes via a seeded watershed based approach.

**Seed locations**

The location of the major pore centres, used as seeds for the watershed transform, can be obtained from the design file or micro-CT volume.
In the f&s file of the design, the octahedron vertices are nodes with the highest degree of connectivity. The centres of major pores respected to a specific axis can be located by shifting the coordinates of all octahedron vertices by half of the unit cell size along the axis direction.

After registration to the design volume, the micro-CT volume of the regular scaffold can be projected onto the three orthogonal directions respectively, as shown in Figure 5.12a & b. Each projected image can be viewed as a topographical map (Figure 5.12c); the basins indicate where interconnecting cylinders (Figure 5.12d) can be inserted into the scaffold. The centres of major pores with respect to a specific axis are represented by the centre of the intersections between all the interconnected cylinders inserted from the remaining two orthogonal directions.

Figure 5.12. Locations of interconnecting cylinders in a regular scaffold; (a) 3D rendered sub volume of regular scaffold image; (b) the sub volume projected on three axis planes; (c) topographical view of the $z$–axis projected image; and (d) location of the interconnecting cylinders inserted from the $z$–axis.
Interconnecting pore segmentation by seeded watershed transform

The interconnecting pores can be segmented by a seeded watershed transform, which is described below:

1. A Euclidean distance transform was applied to the binarised scaffold volume to form a distance map.
2. The distance map was inverted in order for both the voxels of the scaffold phase and all the major pore seeds (with respect to all three axes) to become global minima.
3. Interconnecting pores segmentation was obtained by applying the watershed transform to the inverted distance map. Voxels from each pore were then labelled with different integer values.

Pore size measurement

The equivalent diameter of each pore was calculated to be the diameter of a sphere with equal volume.

5.3.4. Accessible pore size distributions

Unlike regular foam structures, randomised foam structures do not have any form of symmetry (Figure 5.8) which can be segmented with supervision. This is because the coordinates of the octahedron vertices in irregular foams are randomly perturbed and hence there was no clear pattern of octahedron unit within the scaffolds. Therefore, a method termed the accessible pore volume was used to evaluate the pore size distribution in these irregular structures.

This method was designed to mimic the ingrowth of bone and replicate the Mercury Intrusion Porosimetry (MIP) method, in that it calculates the volume fraction of void space within a structure that can be filled by a sphere of a given size, where that sphere could be pushed through connected channels from the surface. This is analogous to measuring the region that mercury can intrude from the surface under a given applied pressure, since the peak pressure in mercury is a function of the curvature cause by the neck.
Figure 5.13. Accessible volume of the UC600 30 % randomised scaffold, (a) 2D representative slice of the binarised scaffold image; (b) distance map; and (c) 2D representative slice of accessible volumes for testing pore with diameter ranging from 80 to 600 µm. In (c), the relative sizes of the testing pore are shown in upper right corner.

The accessible volume for a specific pore diameter was defined as the volume of void space within the region of interest (ROI) that can be accessed by a sphere of that size in a connected manner from the surface of the ROI. The variation in accessible volume was used to estimate the pore size distribution, as follows:

1. A 3D distance map was formed using a Euclidean distance transform of the binarised scaffold image (Figure 5.13a – b).
2. A cylindrical mask, used as region of interest (ROI), was applied to the distance map to remove any voxel outside the scaffold region. Normally the ROI is a cylinder of diameter and height slightly smaller than the scaffold phase in the image.
3. A range of logarithmically spaced vectors was pre-defined to describe the size of the testing pores. For the UC600 scaffold, 11 testing pores with diameter ranging from 80 µm to 600 µm and at equal interval on a logarithmic scale were chosen to balance the quantification accuracy and computational expense.
4. The radius of each testing pore was compared to the numerical value in the distance map, determining the locations in the void space where a sphere of such radius can be placed without overlapping the Ti phase.
5. The voxels calculated along this “accessible path” may not all connect (as determined by a 26 neighbour connectivity) in the void space. Any voxel group which did not connect to the surface of the cylindrical ROI was removed.
6. A dilation algorithm was then applied to the remaining accessible path voxels, using a spherical kernel of the same accessible radius. The volume after dilation is the accessible volume correlated to the testing pore (Figure 5.13c).

7. Step 4 – 6 were repeated for all the testing pores chosen in step 3, providing a range of accessible volumes corresponding to the pore diameter vector.

8. The *accessible volume percentage* was calculated by dividing the accessible volume by the void space volume of the scaffold in the ROI. An *accessible pore size distribution* can then be calculated as the differential of area under the accessible volume percentage curve.

### 5.3.5. Strut roughness quantification

The surface roughness of the struts in a regular scaffold was quantified from the micro-CT volume. When voxelising the regular design, the topology information was integrated into the volume image (as previously described in section 5.3.2). The labelled design volume was used as a ROI to select different strut segments in the micro-CT volume. The local orientation of each strut segment was then calculated via a linear regression analysis using the coordinates of all voxels belonging to the related design strut segment.

Each strut segment was rotated and aligned to an ideal cylinder, which itself is aligned to the vertical axis and represents designed cylindrical struts with a diameter equal to the strut diameter ($D_{\text{strut}}$). $D_{\text{strut}}$ was equal to 180 µm, as estimated previously from SEM images of the regular scaffold.

As shown in Figure 5.14, the lateral surface of the rotated strut segment was extracted and compared to the lateral surface of the ideal cylinder. Direct comparison was made possible by polar transforming the lateral surfaces to planes, so that the difference of the two surfaces could be measured by calculating the distance between the two planes.

The average surface roughness parameter $S_a$ was calculated using the following equation:

$$S_a = \frac{1}{mn} \sum_{k=1}^{m} |Zx_k| \sum_{i=1}^{n} |Zy_i|$$

5.1
Where $Zx$ and $Zy$ are the differences between the actual surface and the reference surface along the $x$ and $y$ directions in the polar transformed planes, where $m$ and $n$ evaluation positions in two axis directions respectively.

Figure 5.14. Polar transformed lateral surface of a strut segment in a regular scaffold. (a) the struts segment was rotated to align to the vertical axis; (b) the lateral surface was un-wrapped using a polar transformation; (c) comparison between (a) and (b) from the top view; and (d) the height difference between the transformed strut segment and the ideal cylinder planes. The surface distance was colour labelled in all sub figures.

5.4. Summary

Porous Ti scaffolds with graded randomness and octahedron unit cell were manufactured by selective laser melting (SLM). The SLM built scaffold were then scanned and quantified using X-ray microtomography (micro-CT). 3D image processing algorithms were developed to quantify the pore network and strut properties of the scaffolds.

These methodologies are applied in the next Chapter to quantify, for the first time, the efficacy of the SLM method in obtaining the designed dimensions, pore distributions and roughness.
6. **Quantification of Selective Laser Melted Titanium Foams for Orthopaedic Applications: Results**

6.1. **Results**

Micro-CT volumes of cylindrical Ti scaffolds with UC600 regular and 30% randomised designs were analysed using the methods described in Chapter 5. Micro-CT volumes of 9 µm voxel size were used for pore network quantifications. The surface roughness of a regular scaffold was analysed using micro-CT volumes of 4.5 µm voxel size.

6.1.1. **Pore size distributions**

Figure 6.1a shows the 3D rendering of the micro-CT volumes of a UC600 regular scaffold. The scaffold was porous with repeated building blocks. Figure 6.1b & c show a sub volume of the regular scaffold and a voxelised design volume respectively. The micro-CT volume was effectively aligned to the design volume through image registration, qualitatively indicating a good agreement between design and built scaffold (shown in Figure 6.1d). At 9 µm voxel size, the scaffold phase in the micro-CT volume was 26% excribed and 18% inscribed by volume compared to the scaffold phase in design image, where *excribed* is the volume percentage laying outside the design volume, and *inscribed* is the volume percentage of the design volume with no material in it in the real build.

The interconnecting pore segmentation was demonstrated on a micro-CT volume of UC600 regular scaffold as shown in Figure 6.2. Three regular samples from a same batch were analysed and compared to the design volume; results are shown in Figure 6.3.
Figure 6.1. 3D rendering of (a) a UC600 regular scaffold and (b) its representative sub volume; (c) is a voxelised design volume and (d) compares the design volume and real scaffold scan by overlapping the two.

Figure 6.2. Segmented interconnecting pores (a) labelled with different colours and also (b) placed into the scaffold structure.
Figure 6.3 shows the interconnecting pore size distributions of regular scaffolds. The equivalent diameter of interconnecting pores was $346 \pm 8 \, \mu m$ for the design volume. The same characteristic for built scaffolds was $330 \pm 14 \, \mu m$, $334 \pm 14 \, \mu m$, and $335 \pm 12 \, \mu m$ for three samples (samples a, b, & c) respectively. The bulk porosity of the three built samples was also measured by voxel number counting and was $63 \%$, $66 \%$, and $63 \%$ respectively, with $0.022 \%$, $0.052 \%$, and $0.025 \%$ of internal (closed) porosity for the three samples respectively.

![Box plots of segmented interconnecting pore size distributions](image)

Figure 6.3. Box plots of segmented interconnecting pore size distributions for the design volume, and three instances of the UC600 regular scaffold.

Figure 6.4 shows the 3D rendering of a micro-CT volume of a UC600 30 \% randomised scaffold. The structure was very porous but no octahedron building unit can be identified.

![3D rendering of a UC600 30 \% randomised scaffold](image)

Figure 6.4. 3D rendering of (a) a UC600 30 \% randomised scaffold and (b) its internal structure.
The accessible pore size distributions of a UC600 regular scaffold and a 30% randomised scaffold were analysed and compared to the pore size distributions previously (Mullen et al., 2010) obtained by Mercury Intrusion Porosimetry (MIP), as shown in Figure 6.5.

![Figure 6.5](image)

Figure 6.5. Pore size distributions of a UC600 regular and 30% randomised scaffolds obtained by (a) accessible volume percentage (b) Mercury Intrusion Porosimetry.

In terms of accessible volume, 97.1% of void space in the regular scaffold can be accessed by a testing pore with a diameter of 80 μm. This percentage gradually decreases to 84% as the testing pore diameter increases to 178 μm. The accessible volume percentage suddenly drops to 42.6% at 218 μm diameter size, and further decreases to 8.6% at 266 μm diameter size. Almost no space can be accessed by a testing pore with a diameter greater than 396 μm. For the irregular scaffold, the volume percentage decreases more gradually as the pore diameter increase, and also has wider range. The percentage of accessible volume by larger testing pores in irregular scaffold is greater than which in regular scaffold, make the structure easier for bone ingrowth and blood vessel penetration. This is due to the perturbation of octahedron vertices coordinates contributed in forming larger pores and pathways in the irregular structure.

The modal diameters for derived pore size distributions were 217 μm and 324 μm for regular and irregular scaffold respectively (Figure 6.5a). Using MIP data (Figure 6.5b), the estimated pore diameters modes were 225 μm and 281 μm for the regular and irregular scaffolds respectively.
6.1.2. Surface roughness

The strut segment roughness of a UC600 regular scaffold was quantified from a high resolution scan volume. The \( S_a \) values of 60 strut segments are plotted in Figure 6.6, with an average value of 22 ± 4 \( \mu \)m.

The surface roughness of a UC600 regular scaffold was qualitatively compared between micro-CT volumes of 4.5 \( \mu \)m and 9.06 \( \mu \)m voxel sizes, as shown in Figure 6.7. In Figure 6.7a & c, the sub volume of the high resolution scan containing four octahedron unit cells is rendered as an isosurface. Compared to Figure 6.7b & d, which are isosurfaces of a similar block from low resolution scan, there are many more surface details in Figure 6.7a & c. It can also be clearly seen that in high resolution volumes, the top side surface (Figure 6.7a) of the scaffold was rougher than the bottom side surface (Figure 6.7c). Such an observation was not clear in the low resolution volume (Figure 6.7b & d).

The difference between top side and bottom side surfaces of the regular scaffold in the high resolution scan (4.5 \( \mu \)m voxel) was quantified and is shown in Figure 6.8. Figure 6.8 a & b are top side and bottom side surfaces respectively of a scaffold block containing four octahedron unit cells. The sub volume was then smoothed by morphological opening (erosion followed by dilation): the top side and bottom side surfaces of the smoothed sub volume are shown in Figure 6.8 c & d. The distance between the original surface and the morphologically smoothed surface are shown in Figure 6.8 e & f: the distance is rendered with pseudo colour.
Figure 6.6. $S_\alpha$ profiles of 60 strut segments in a UC600 regular scaffold.

Figure 6.7. Effect of scanning resolution on surface roughness detail. (a) and (c) show the top side and bottom side respectively of a sub volume in UC600 regular scaffold scanned at 4.5 µm voxel size; (b) and (d) are similar renderings to (a) and (c) respectively obtained from a 9.06 µm voxel size scan. The size of the featured ROI was 1.2 mm by 1.2 mm by 0.6 mm.
Figure 6.8. Orientation dependent local surface roughness in a UC600 regular scaffold. (a) and (b) are 3D isosurface rendering showing the top side and bottom side respectively of four octahedron unit cells in a regular scaffold; (c) and (d) shows the top side and bottom side respectively of the scaffold volume after morphological smoothing; (e) and (f) show the colour labelled distance between surfaces of original image and smoothed image, with the same viewing angle as in (a) and (b) respectively. The colour bar indicates a distance difference from 0 to 25 µm.
6.2. Discussion

This study uses high resolution X-ray microtomography (micro-CT) as a non-destructive imaging tool to evaluate Ti scaffolds with graded randomness which were made by selective laser melting (SLM). The Ti scaffolds were designed to function as orthopaedic fixation devices which need to integrate with the host tissue through bone ingrowth after implantation. Therefore possession of a porous structure with preferable pore size distribution, porosity, and bulk modulus is vital to the success of such implants. Here, micro-CT coupled with 3D image processing techniques has been demonstrated as a powerful combination to characterise porous scaffolds.

6.2.1. Voxelisation and registration

A design volume which represents the intended scaffold design was created by voxelisation using the locations of laser melting spots as input. The coordinates of laser spots provided by the SLM input files (f&s files) were used as prior knowledge to build the ideal design volume. This voxelisation method effectively simulated the building mechanism of SLM, with controllable strut diameter. Overlay of design and sample volumes showed differences of 26% excribed and 18% inscribed. This difference appears to be attributable to the quality of the surface brought about by the effects of attached Ti particles (inherent in the process) rather than by variability initiated by machine process control. Additionally, the strut morphology in real sample was affected by layered building process and post sintering, and differs from which in the design volume. This difference affected the accuracy of image registration and therefore resulted in the difference in overlaid volumes. The amount of excribed volume was closed to the amount of inscribed volume, indicating the assumed strut diameter of 180 µm in design volume was well matched to the average strut diameter of the real productions.

The strut diameter, strut morphology, and surface roughness of the design volume can be controlled by using a different convolving kernel to better represent the reality. For example, Figure 6.9c shows a voxelised volume using a half-sphere as the kernel for convolution. In the ideal case, a spherical convolving kernel was used, and thus the process was simplified to an isotropic dilation based method. This method also made it possible to classify and label the scaffold struts into different groups according to their topological roles, and provided information to calculate surface roughness.
The stereolithography models (the STL files) were not used to directly create the virtual model because of tolerance issues brought about by the surface tessellation of the original CAD data; and also the fact that the STL data was not used in directly building the parts on the SLM machine.

### 6.2.2. Pore network quantification

After implantation of orthopaedic scaffolds, the local bone will regenerate and grow into the porous structure and form a mechanical interlock, providing stable attachment by osseointegration. The porosity, pore size distribution, and pore morphology of the scaffold will greatly affect the success of the fixation by affecting solute transport, blood vessel regeneration, and bone ingrowth. In this study, the pore networks of the Ti scaffolds were quantified by means of explicit segmentation as well as accessible volume method.

The regular scaffolds possess a repeating octahedron unit cell. This octahedron pattern was represented by interconnecting pore segmentations. The interconnect cylinders defined in Mullen et al. (Mullen et al., 2009) were similar to a stack of interconnecting pores. However, because the interconnecting pore segmentation filled the entire void space in the volume, the equivalent pores diameter length ($330 \pm 14 \mu m$) was greater than the theoretical diameter length of the interconnecting cylinder ($244 \mu m$). This is also the main reason why the average equivalent diameter of the interconnecting pores was larger than the modal accessible pore diameter ($217 \mu m$) for the same regular scaffold.

Using interconnecting pore segmentation, the difference between the pore diameters of the SLM built sample and design volume was only determined by true strut morphology and roughness; interconnecting pore segmentation can thus be used as a tool to evaluate the production quality of the scaffold as the segmentation is unique for a particular scaffold. In Figure 6.3, the interconnecting pore size distribution had a greater modal value and narrower distribution compared to the real values. This was because the pore size variation in the design volume was only caused by the voxelisation accuracy and half voxel rounding effect. In real scaffolds, smaller modal pore size and wider distributions were also caused by the complex surface morphology of the strut.

In contrast to having a well defined repeating building pattern in the regular design, no clear building unit can be identified in 30% randomised scaffolds. In those scaffolds the pore
network was characterised by the accessible volume method. This process is similar to that of Mercury Intrusion Porosimetry (MIP), but has a discrete pore diameter range. The test pore shape was set to be spherical, which implies that the results will be strongly affected by strut surface roughness and voxel size. For example, the size of the largest pore in the void space can be reduced by only one small fluctuation at the strut surface; also a larger voxel size will practically smooth the surface of the scaffold and also affect the shape of test spheres (especially with regards to small spheres).

Due to the computational limitations, a discrete range of pore diameters was used. Increasing the number of test diameters would give more accurate results, but would also be more computationally expensive. The test pore diameters were chosen empirically by, considering the pore size obtained from the interconnecting pore segmentation of the regular scaffold with the same unit cell size.

The results obtained by the accessible volume method agree well with the MIP data in terms of modal values and distribution shapes for both regular and irregular scaffolds. This indicates that the destructive MIP testing can be replaced by micro-CT coupled with image processing technique for these scaffolds. However, the accessible volume method was only appropriate when the structure was highly porous and open. This model will not be suitable when larger pores are connected by significantly narrower interconnects such as the structures of bioactive glass foams. In such structures, the pore size distributions obtained by accessible volume method actually reflect the interconnect size distributions.

The quantitative results show that the SLM built UC600 scaffolds, with either regular design or irregular design, all have pore size distributions in the preferred range for human bone ingrowth, i.e. with modal values far greater than 100 µm.

6.2.3. Surface roughness quantification

Selective laser melting (SLM) can produce parts directly from computer model. The building quality, however, is strongly affected by the Ti particle properties (size distribution, thermal properties), the machine settings (laser beam size and energy, step size), as well as the geometric characteristics of the design.
For a given exposure time, the effective laser melting kernel was affected by the combination of thermal properties of the raw Ti powder and the laser beam properties. In this study, the direct laser melting kernel was assumed to be spherical and with a diameter $D_{\text{melting}}$ equal to 180 $\mu$m. This assumption simplified the consolidation kinetics while still reflecting the SLM building mechanism.

![Diagram of laser beam melting process](image)

Figure 6.9. Roughness caused by stair-stepping in the SLM building process. (a) – (c) show the 2D schematic of the layer-wise build process of a single edge in regular scaffold; (d) is a created volume when considering the layer effect by using a half sphere convolving kernel; and (e) the location of the laser melting spots from the f&s file.

The Ti powder was applied to the powder bed in 50 $\mu$m thick layers. The Ti particles in the current build layer were fused by laser beam (Figure 6.9a). The thermal energy of subsequent laser beam scans is typically sufficient to re-melt a portion of layered solidified structure underneath (Figure 6.9b & c). The effective laser melting window was thus a half sphere, as illustrated in Figure 6.9.

This intrinsic property of the SLM building process caused the difference in local surface roughness between top side and bottom side of the scaffold, as shown in Figure 6.7 and Figure 6.8. The Ti particles forming the bottom side of scaffold were fully melted by the half spherical isothermal kernel, leaving a good built surface. The top side of the scaffold,
however, possessed a “stair-steps” texture resulting from the layer building process. Stair-stepping is a fundamental issue in layered manufacturing and is difficult to overcome (Gibson et al., 2009). Its effect can be minimised by choosing a thin layer thickness and an adequate post-thermal processing.

After SLM building and cleaning, parts are thermally processed in a vacuum oven 1400 °C for three hours, to enhance their properties to give the desired microstructures and/or to relieve residual stresses. This process also allows the partial smoothing of the scaffold surface by filling in the stair-step features with the melted adhered particles, and also brings about a reduction in the surface energy (which is proportional to the surface area) as the surface area is consequently reduced (Figure 6.10).

![Figure 6.10. SEM images showing (a) unsintered and (b) three-hour 1400 °C sintered SLM wire-frame struts (Courtesy of Eric Jones, Stryker Orthopaedics).](image)

The $S_a$ values quantified for the regular scaffold reflect the difference between the produced scaffold and the intended design, but do not reflect the local roughness. In the current model, the strut segments in the intended design were assumed to be cylindrical without considering the stair-stepping effect. Additionally, this cylindrical surface of the intended strut design was used as the reference surface for roughness quantification. This method differs from other surface roughness quantification techniques which estimate the reference surface using Gaussian smoothing based methods.

In the real scaffolds, the laser spots at or near the vertices in the octahedron network melted and rounded into/with each other; and it was exaggerated after post-thermal processing of SLM built scaffold. Hence, only the struts segments were used to calculate the $S_a$ profiles.
The details of the surface texture were directly affected by the resolution of the micro-CT scan. A fine resolution is essential to accurately represent the geometry in a discrete space. When un-wrapping the lateral surface of the strut segment, the angular difference was chosen so that the length of the un-wrapped surface of an ideal cylinder equal to its perimeter. Therefore the obtained $S_a$ values can also be affected by the accuracy of strut diameter estimation.

The accuracy of the surface roughness quantification was mainly affected by the quality of volume registration and resolution of the micro-CT scan. The algorithms used here assumed that the framework of the built scaffolds aligned well with the design volume. Distortion of the scaffold possibly caused by thermal treatment or built error would result in poor local alignment, thus producing a large $S_a$ value. Therefore the $S_a$ derived from this work can be used to act as a quality control indicator for SLM built scaffolds.

6.3. **Summary**

Pore size distributions and surface roughness profiles for SLM built UC600 scaffolds were quantified from micro-CT scans using the methods presented in Chapter 5.

Quantitative results indicate that UC600 scaffolds with regular and irregular designs all have suitable pore size distributions and porosity levels for human bone ingrowth. The average porosity of the real scaffolds was 64 ± 2 %, which is consistent with the intended porosity of 65 %. The regular structures were explicitly segmented and had a modal interconnecting pore size of 330 ± 14 µm. Pore size distributions obtained by the accessible volume method agreed well with MIP results, with modal pore diameters of 217 µm and 324 µm for regular and irregular scaffolds respectively.

Image registration results show good agreement between the ideal design and the real production of a UC600 regular scaffold. Additionally, the area surface roughness profile, the $S_a$ value, which also acted as a quantitative indicator of built quality, was 22 ± 4 µm for the UC600 regular scaffold.
7. CONCLUDING REMARKS

As a result of a rapid increase in life expectancy since the 20th century, maintaining the quality of life for the ageing population has become a challenge in public healthcare. One particular need is to repair aged, diseased and damaged bone, which is currently the second most transplanted tissue after blood. The traditional methods to treat critical bone loss, such as autologous and allogeneic transplantations, cannot meet the requirements in terms of availability and quality. Tissue engineering, which employs the concept of regeneration instead of replacement, is considered as a promising approach to tackle the challenge. However, the subject remains an inexact science.

Scaffold design is an important aspect in tissue engineering. In order to act as a template and guide for tissue regeneration, the scaffold needs to be chemically bioactive and friendly to the body, as well as in possess of a preferable porous structure.

In this thesis, the use of X-ray microtomography (micro-CT) for scaffold characterisation has been highlighted. Micro-CT, which can non-destructively image the internal structure of the scaffold, has been found a powerful tool when coupled with advanced image processing techniques. The research in this thesis has focused on transforming the micro-CT visualisations into quantitative data for the design and analysis of scaffolds.

New methods for porous structure segmentation and quantification from 3D micro-CT images were developed and which are able to work for a range of porous structure, including:

- Melt-derived bioactive glass scaffolds produced by gel casting;
- Sol-gel derived bioactive glass scaffolds;
- Ti scaffolds produced by selective laser melting (SLM).

Using these novel techniques, the following new insights were obtained from this thesis:
A 3D image processing method has been developed which can accurately quantify the structure parameters of bioactive glass scaffolds.

- Both melt-derived and sol-gel derived bioactive glass scaffolds have suitable pore and interconnect size distributions as bone scaffold. With the newly developed method, the quantified modal equivalent pore diameter and interconnect diameter for melt-derived scaffold were 425 µm and 128 µm respectively. Sol-gel derived scaffold had larger pore and interconnect sizes, with modal equivalent pore and interconnect diameters of 682 µm and 252 µm respectively.

- The accuracy of interconnect size quantification has been significantly improved from previous studies by using a principal component analysis (PCA) algorithm to locate the natural orientation of each interconnect; the size of the interconnect can then be effectively and efficiently measured using its 2D projection onto the principal plane in an automated fashion. In this way, the interconnect size distribution obtained for a scaffold is based on the explicitly calculated equivalent diameters; whereas previously a global correction factor obtained by manual measurement must be applied to the interconnect size distribution obtained from the bounding box method in order to avoid overestimation.

- The dissolution of a 70S30C bioactive glass foam in SBF perfusion flow has been quantified for the first time using micro-CT. Heterogeneous degradation and redeposition were observed and the variation of size distributions were measured with the newly developed method. While the pore size distribution remained almost unchanged with a modal diameter of 682 µm during the 28-day dissolution period, the modal interconnect diameter reduced from 252 to 209 µm due to mineral deposition. This also highlighted the advantageous non-destructive nature of micro-CT for scaffold characterisation.

- The structure quantification method has been extended to work for SLM built scaffolds.

- Explicit segmentation was obtained for Ti scaffolds with a regular pore structure and the accessible volume method was developed, which can be applied to both regular and irregular structures. For the UC600 regular scaffold, the average interconnecting pore size was 330 ± 14 µm. Using the
accessible volume method, the modal pore diameters for UC600 regular and 30% randomized scaffold were 217 µm and 324 µm respectively, which agreed well with MIP results.

- A method to quantify surface roughness profile of SLM built scaffolds was developed. The area surface roughness profile, $S_a$, was $22 \pm 4 \mu m$ for the UC600 regular scaffold.

- Using the developed voxelisation technique and image registration technique, it can be seen that the manufactured regular scaffold agreed well to the design volume, with 26% excised difference and 18% inscribed difference in voxel number counting.

- The method can be extended to other additive manufactured parts and can act as a quality control (QC) and quality assurance (QA) tool.
8. **FUTURE WORK**

The research in this thesis has developed a new method for structural quantification of tissue scaffolds from micro-CT images and the technique was applied to quantify several scaffolds ranging from bioactive glass to SLM built Ti foams. According to the results of this study and conclusions drawn, the following recommendations are proposed for the future work.

- The image based quantification technique was able to segment the pore network to macro pores and interconnects, and then provide quantitative statistics such as pore and interconnect size distributions. The size and connectivity of segmented pores and interconnects (as shown in Figure 3.9), can be used as the input for pore network analysis for flow simulation (Dong and Blunt, 2009).

- In Chapter 4, the dissolution study of sol-gel derived 70S30C bioactive glass scaffold was using SBF as the flow medium and was focused on looking at the impact of dynamic flow on the dissolution properties of the 3D scaffold. However, the use of SBF to predict apatite formation was not ideal. Additionally, the perfusion bioreactor used in this study was very simple and was not optimised for in vitro cell seeding on the scaffold. Therefore, a more advanced dissolution experiment would use physiological fluid such as culture medium to optimise the scaffold design in terms of in vitro tissue formation.

- Image based finite element analysis and computational fluid dynamics can be incorporated into the quantification algorithms. Using the simplified pore network, the amount of computational work can be significantly reduced. Therefore a standalone, rapid inspection tool can be provided to the research community.

- Using the developed 3D quantification algorithms and based on the non-destructive nature of micro-CT, the characterisation techniques can be extended to in vivo studies. Parameters such as bone ingrowth rate, scaffold degradation rate, and blood vessel penetration rate, can be quantified from ex vivo sample to investigate the in vivo performance of the scaffold.
In addition to bone ingrowth quantification, the mechanical properties of the scaffold before and after implantation, as well as a portion of trabecular bone, can be compared using micro-CT based *in situ* compression experiments. The failure mechanism and local strain rate can be observed and quantified from a series of micro-CT images taken at different stage of the compression.

The strut roughness in Ti scaffold can greatly affect the cell adhesion response and its solute transport property. In additive manufacturing, strut segment with different building angle have varying surface profile. In order to optimise the SLM scaffold design, this impact of this phenomenon can be studied by comparing the real productions with different building angles to simulated results.

The technique used for SLM built Ti scaffolds quantification can be extended to act as a quality assurance (QA) and quality control (QC) tool. Such method is required for FDA approval of the products used in health care.
9. **APPENDIX A**

The Matlab codes developed in this thesis for 3D image processing are included in an attached DVD and the core functions are listed in Table 9-1 with brief summaries. The codes were developed under Matlab 7 environment and have been tested on both Windows and UNIX platforms with Matlab versions from R2008a to R2011a, and Image Processing Toolbox versions from 6.1 to 7.1. Detailed usage can be found in the documentation of each function.

Table 9-1. Matlab codes for pore structure quantification.

<table>
<thead>
<tr>
<th>Name</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>readraw.m</td>
<td>Read volume files into workspace</td>
</tr>
<tr>
<td>labelLargest.m</td>
<td>Locate the largest connected object of scaffold phase</td>
</tr>
<tr>
<td>apraw3.m</td>
<td>Perform PCA based interconnect size quantification</td>
</tr>
<tr>
<td>estimateDistribution.m</td>
<td>Estimate the size distributions weighted by volume or area</td>
</tr>
<tr>
<td>plotAreaFraction.m</td>
<td>Plot area fraction graph for interconnect size distribution</td>
</tr>
<tr>
<td>plotVolumeFraction.m</td>
<td>Plot volume fraction graph for pore size distribution</td>
</tr>
<tr>
<td>FnS2Volume.m</td>
<td>Convert f&amp;s files into volume files</td>
</tr>
<tr>
<td>locateSeeds.m</td>
<td>Locate the pore centres in regular Ti scaffold for watershed</td>
</tr>
<tr>
<td>regularQuan.m</td>
<td>Quantify the pore network in regular Ti scaffold</td>
</tr>
<tr>
<td>accessibleVolume.m</td>
<td>Quantify pore size distribution by accessible volume</td>
</tr>
<tr>
<td>regularRoughness.m</td>
<td>Quantify the surface roughness profile for regular scaffold</td>
</tr>
</tbody>
</table>

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10. REFERENCES


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