PROTOCOL DEVELOPMENT FOR THE ANALYSIS OF BIPHASIC CALCIUM PHOSPHATE SCAFFOLDS AS A FUNCTION OF DISSOLUTION USING MICRO-CT ANALYSIS
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Introduction
Optimising dissolution rate of calcium phosphate (CaP) scaffolds is necessary to ensure that CaP scaffolds allow total tissue regeneration within a repair site while maintaining structural support long enough to allow repair to progress [Blom, 2007]. However, conventional measurement of dissolution [Ho and Hutmacher, 2006], e.g. by gross scaffold mass loss, lacks the detail necessary to fully understand the influence of dissolution on structural strength. The aim of this study is to develop a protocol to measure dissolution of CaP scaffolds using micro-computed tomography (µCT) analysis. In future, data generated using this protocol will be used to steer development, and corroborate predictions, of a computational model of CaP scaffold dissolution.

Methods
The protocol was developed using existing µCT scans of biphasic hydroxyapatite and beta tri-calcium phosphate scaffolds (50:50 wt. %) [Newe, 2012]. Scaffolds with a trabecular structure were created using polyurethane foam as a pre-cursor material [Newe, 2012]. Dissolution was quantified at different time points (day 0, 1, 3, 5) of accelerated dissolution within a static medium (pH 4) [Newe, 2012]. The µCT scans were registered using a rigid registration algorithm within Fiji [NIH, 1997] which placed all of the scans in the same orientation. Scans were also thresholded in order to remove background noise. A detailed understanding of the dissolution process was acquired by considering global, regional, and local scales (Figure 1).

\[ \text{Figure 1: Global, regional and local measurements of scaffold dissolution} \]

Bone volume decrease was calculated globally (Figure 1a.) and compared to conventional mass loss data. A similar analysis compared central and peripheral regions of interest (Figure 1b.) and their differences in dissolution. Local measurements considered single trabecular structures (Figure 1c.) and were used to evaluate if dissolution occurred as a bulk process or from the surface material.

Results
Bone volume decrease was found using µCT analysis and was comparable to conventional mass loss data (Figure 2) on a global scale. Regional measurements for trabecular spacing/pore size during dissolution increased at day 1, but decreased at day 3 and 5 (Figure 3). The grey value at the centre of a trabecular structure decreased as a function of time in dissolution (Figure 4), indicating dissolution occurred throughout the trabecular thickness.

\[ \text{Figure 2: Percentage decrease in bone volume as a function of time in dissolution} \]

\[ \text{Figure 3: Mean trabecular spacing for a scaffold as a function of time in dissolution (standard deviations correspond with values from the five regions of interest in Figure 1b)} \]

\[ \text{Figure 4: Grey value against distance along a trabecular structure (high grey values indicate dense material and a change in peak indicates a decrease in material)} \]

Discussion
A protocol was successfully developed for measuring dissolution of CaP scaffolds using µCT analysis, which provided more detail compared to conventional approaches. The protocol was shown to be comparable on a global scale (Figure 2) to experimental results. Greater detail was available at regional levels (Figure 2). However, these results may be unreliable because of an increased likelihood of precipitate formation at day 3 and 5 indicated by an unexpected decrease in average pore size. Precipitate formation was also evident on a local scale; this is shown in Figure 4b as day 1 and 3 plots “shift” to the right of the graph indicating an increase in density. Establishing the mode of dissolution was possible using grey value plots of trabecular structures, as a change in peak indicates loss at the centre of the structure (Figure 4), but further work will be required to make definitive conclusions for dissolution mode.

References