



# X-ray microtomographic analysis of $\alpha$ -tricalcium phosphate-poly(lactic-co-glycolic) acid nanocomposite degradation



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## ARTICLE INFO

### Article history:

Received 21 March 2014  
Received in revised form  
23 May 2014  
Accepted 1 June 2014  
Available online 19 June 2014

### Keywords:

Poly(lactic-co-glycolic) acid  
Calcium phosphate  
Nanocomposite

## ABSTRACT

The degradation characteristics of  $\alpha$ TCP-PLGA(50:50) nanocomposites containing varying ceramic weight loadings in an aqueous medium have been assessed using X-ray microtomography ( $X\mu$ T). Also measured were bulk density changes, pharmaceutical drug release and medium acidification for the degrading materials.

Calcium phosphate addition to the polymer leads to increasing delays in the onset of degradation medium acidification and tetracycline release. Bulk density changes with time for all composite materials measured using a buoyancy method were well described during the initial degradation regime by a  $t^{1/2}$  function. PLGA density evolution follows a linear function of time which indicates a differing water absorption process occurring in the pure polymer compared with the nanocomposites.

Nanocomposite microtomographic analysis over the same period elucidated a core-periphery structure caused by water imbibition. Peripheral regions closest to the specimen surface exhibit reduced attenuation coefficients compared with the core which may be characteristic of a frontal system caused by a polymer phase transition. The front position and specimen swelling are adequately described by a  $t^{1/2}$  and complementary error function respectively which if assessed under the assumption of a diffusion controlled process yields a diffusion coefficient of water in all nanocomposites at 37 °C of  $4.8 \times 10^{-14} \text{ cm}^2 \text{ s}^{-1}$ . Nevertheless, a  $\sqrt{t}$  dependence is a necessary but not sufficient condition of a Fickian diffusion process. For all nanocomposite types both  $X\mu$ T data and bulk density measurements exhibited no variations with ceramic filler content.

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## 1. Introduction

Composites composed of nanoparticulate  $\alpha$ -Tricalcium phosphate evenly dispersed in poly(lactic-co-glycolic) acid (50:50 monomer ratio) are candidate materials for use in resorbable orthopaedic devices.

The investigation of bioceramic nanocomposites is motivated by the weight of evidence supporting the biological and physical enhancement of biodegradable polymers by nanoparticulate incorporation compared with equivalent microcomposites. Properties enhanced by nanoparticle addition include greater material moduli, suppression of autocatalytic degradation and cytotoxicity by acid degradation product neutralization. A review of the

rationale and evidence for the aforementioned improvements to PLGA properties by  $\alpha$ TCP incorporation was performed by Barrett et al. [1]. Additionally, preliminary evaluations of  $\alpha$ TCP-PLGA nanocomposite physical property changes with degradation time may be found in the works of Yang et al. [2] and Ehrenfried et al. [3] with further complementary biological characterization of the degrading materials by Meyer et al. [4].

The absence of systematization in the assessment of degradable polymer-ceramic systems has been identified by Vert [5] as a salient problem in modern biodegradable material investigation. The principal impediment to biomaterial degradation property evaluation is the small length scales associated with degradation phenomena which limits the effectiveness of conventional characterization methods.

This article details characterization of the degradation behavior of pure PLGA(50:50) and  $\alpha$ TCP-PLGA(50:50) containing 10, 20, 30 and 40 wt%  $\alpha$ TCP nanoparticles. X-ray microtomographic analysis is used to evaluate internal morphological changes as functions of

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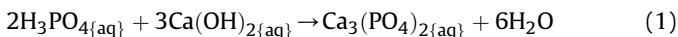
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degradation time to assess the effects of water imbibition. In parallel, degradation medium acidification measurements, tetracycline drug release data and specimen bulk density changes measured using a buoyancy technique are presented to systematically elucidate the degradation kinetics of the nanocomposite materials.

## 2. Sample preparation

### 2.1. Materials

Nanoparticle  $\alpha$ TCP was synthesized at room temperature using an aqueous precipitation reaction according to



Aqueous 0.187 M calcium hydroxide (Sigma Aldrich, UK) and 0.121 M phosphoric acid (analytical reagent grade, BDH Laboratory Supplies, UK) were stirred separately for 1 h then mixed together and stirred for a further hour in order to homogenize the solution. The solution was left to age for 24 h then filtered leaving a gelatinous paste. After evaporating the water in a drying cabinet the solid was crushed with a mortar and pestle and ball milled for 4 h. The dried powder was sieved using a 75  $\mu\text{m}$  mesh followed by sintering at 1400  $^\circ\text{C}$  for 4 h after which the sample was cooled rapidly to room temperature.

TCP polymorph phase identification was performed using an X-ray powder diffractometer (Philips X-Pert PW3020 vertical diffractometer) employing  $\text{Cu K}\alpha$  X-rays operating at source voltage and current settings of 40 kV and 40 mA. Diffraction patterns were acquired over the angle range 10 $^\circ$  – 50 $^\circ$  using a step size of 0.02 $^\circ$  and dwell time of 30 s.

Post-acquisition, quantitative peak fitting was performed using the software Highscore Plus employing the reference intensity ratio method (RIR) to assess the material phase content. Acquired powder diffraction patterns were matched to material reference diffraction data from the International Centre for Diffraction Data (ICDD), selecting the patterns corresponding to  $\alpha, \beta$  TCP, and HA in addition to other commonly occurring compounds in calcined materials such as calcium oxide. The RIR method determined that the phase purity of the TCP was >95% which meets the required criterion for polymorph phase purity.

The  $\alpha$ TCP crystal size was assessed using peak broadening analysis of X-rays diffracted from a crystal of width  $d$  using the same Phillips diffractometer system according to the Scherrer formula

$$d = \frac{K\lambda}{\gamma\cos(2\theta)} \quad (2)$$

where  $\gamma$  denotes the crystal diffraction peak full-width half maximum,  $K$  is a geometrical constant,  $\lambda$  the X-ray wavelength measured in nanometres and  $2\theta$  the Bragg angle (in radians). An intermediate value of the Scherrer constant of 0.9 was used in although this may deviate significantly from this value for some crystal shapes [6]. The instrument mediated peak broadening as a function of scattering angle was determined by the linear interpolation between instrument broadening measured for large crystal silicon which produces diffraction peaks at 28.3 $^\circ$  and 47.2 $^\circ$  neglecting possible non-linearity of diffraction profiles with Bragg angle. The Scherrer analysis demonstrated that the crystal axes corresponding to the most intense diffraction peaks were in the 20 – 50 nm range.

Nanocomposites were formed using a solvent evaporation method with prior attritor milling in acetone for 4 h to reduce the TCP particle size. The material was formed into nominally 900  $\mu\text{m}$

thick sheets by compression molding. Specimens in the form of disks of diameter 8 mm were cut from the sheet using a circular punch. The high diameter to thickness aspect ratio was chosen to limit edge effects in order that material swelling and water absorption phenomena would primarily occur along the disk axis to facilitate degradation phenomena assessment in one dimension only.

Ceramic particle sizes in the composites were measured by Wilberforce et al. [7] using a cold cathode field emission electron SEM (S-5500, Hitachi, Japan). Samples differed from those studied in this investigation in that injection molding rather than compression molding was used in specimen manufacture. The modal particle sizes for 10, 20 and 30 wt% composites is below 150 nm with relatively few particles exhibiting dimensions greater than 500 nm. Agglomeration and a significantly greater particle size dispersion is observed for 40 wt% composites which also possess a significant percentage of particles with dimensions greater than 500 nm. Nonetheless, the distribution indicates that a nanocomposite and not a microcomposite is formed using the attritor milling and compression molding procedure.

Specimens manufactured for the tetracycline drug release study were made using the same solvent evaporation technique detailed above with the sole exception that approximately 0.2 g of tetracycline per 20 g of composite was added to the slurry 30 min prior to completion of attrition milling.

### 2.2. Degradation study

Samples were degraded in 40 ml distilled, deionized water (sample to water volume ratio 1:900) at 37.4  $^\circ\text{C}$  in PTFE containers for various times in order to assess composite morphology as functions of degradation time.

Aqueous medium acidity measurements were performed at regular intervals using an electronic pH meter (HI-98230, HANNA Instruments) which was calibrated prior to each testing series using standard solutions. The variation between specimens was determined to be greater than individual time point measurement errors, therefore the uncertainty associated with each material at each time point was calculated as the standard deviation between specimens of the same composition.

For specimens containing the pharmaceutical, tetracycline concentrations were evaluated at regular intervals using a UV spectrometer (Permer–Elkin) calibrated using tetracycline peak absorption at 273 nm. Possible concerns regarding calcium ions influencing tetracycline measurements were evaluated by assessing the effect of known concentrations of aqueous calcium hydroxide on the tetracycline chromophore; no variations in measured tetracycline concentrations with increasing calcium concentration were observed.

Density measurements were performed using a water buoyancy method calculating the specimen bulk density according to

$$\rho = \rho_0 \frac{m_{\text{air}}}{m_{\text{air}} - m_{\text{water}}} \quad (3)$$

At regular intervals specimens were removed from the degradation study and the mass measured in water,  $m_{\text{water}}$ , using a modified balance simultaneously recording the water temperature using a thermometer from which the water density,  $\rho_0$ , was determined from a look-up table. Specimens were then dabbed dry and mass in air,  $m_{\text{air}}$ , measured.

## 3. Tomographic analysis

The X-ray microtomographer used in this investigation is a SkyScan-1072 (SkyScan, Aartselaar, Belgium). X-rays are generated

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