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Degradation and drug release in calcium polyphosphate bioceramics: An MRI-based characterization

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ABSTRACT

Degradable, bioceramic bone implants made of calcium polyphosphate (CPP) hold potential for controlled release of therapeutic agents in the treatment of localized bone disease. Magnetic resonance imaging techniques for non-invasively mapping fluid distribution, T_1 and T_2 relaxation times and the apparent diffusion coefficient were performed in conjunction with a drug elution protocol to resolve free and bound water components within the material microstructure in two CPP formulations (G1 and G2). The T_2 maps provided the most accurate estimates of free and bound water, and showed that G1 disks contained a detectable free water component at all times, with drug release dominated by a Fickian diffusion mechanism. Drug release from G2 disks was characterized by a combined diffusional/structural relaxation mechanism, which may be related to the gradual infiltration of a free water component associated with swelling and/or chemical degradation.

1. Introduction

With the increasing use of biomaterials for tissue regeneration and controlled drug release, there is a greater need for characterization techniques that can assist with the optimization of these materials. In particular, biodegradable implant materials represent highly dynamic systems that require a non-invasive approach to fully understanding the fundamental mechanisms of degradation and drug release. Magnetic resonance imaging (MRI), in addition to being a non-invasive tool, offers a range of image contrast mechanisms that are sensitive to the mobility characteristics of sorbed water, and provide an endogenous probe of the chemical and physical microstructure. MRI is therefore a powerful technique for characterizing degradable biomaterials and for understanding their function, as well as potentially speeding their development as effective therapeutic options [1,2].

The present authors have developed an MRI protocol for studying microstructural changes in degradable calcium polyphosphate (CPP) bioceramics. CPP is a form of condensed phosphate, which is sometimes referred to as an "inorganic polymer" [3,4]. The combination of ceramic and polymeric characteristics in CPP make it a potential alternative to other biopolymers, with manipulation of drug release possible through the physico-chemical reactions to water (i.e. chain scission, gelling/swelling, and bulk erosion [3,5,6]). Because of these characteristics, implantable devices made from amorphous CPP have been explored for controlled-rate drug release in localized therapy [7–9], specifically for the delivery of antibiotics in the treatment of chronic, localized bone infections (osteomyelitis) [7,8,10–12]. Furthermore, biodegradable materials such as CPP have the attractive quality that no surgery is required to remove the device, which is beneficial because it reduces both patient discomfort and the risk of reinfection during surgery [13–15].

In a material such as CPP, the goals of controlled-rate release and biodegradation are related, because drug is eluted from the device through fluid absorbed from the environment (i.e. the body), and the fluid transport is, in turn, governed by the physical and chemical microstructure of the implant material. As such, a mechanistic understanding of the relationship between microstructural transformation and drug release behavior is crucial, and must be obtained in a way that does not disrupt the dynamics of the system [16]. Previous research into the structure/elution characteristics of CPP drug delivery bioceramics has been carried out using techniques limited to the study of "dry" materials (e.g. scanning electron microscopy) and to bulk methods averaged over the entire material (e.g. drug elution aliquots). What is lacking is an understanding of how the fabrication parameters influence the microstructure, how local degradation occurs and, ultimately, how this affects rates of release.

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MRI techniques permit measurement of parameters that are sensitive to the mobility of imbibed water in a non-invasive manner, making possible the observation of the time-evolved behavior. While a large number of MRI contrast mechanisms exist, three of the most commonly used are spin–lattice relaxation (T_1), spin–spin relaxation (T_2) and the apparent diffusion coefficient (ADC) [17,18]. Each is sensitive to molecular mobility (e.g. tumbling frequency, diffusive translation) on different spatial and temporal scales, and is generally a process involving exponential signal changes that can be characterized with an associated exponential constant. Using standard imaging techniques, it is possible to isolate and map these parameters, which in turn reveal spatially varying characteristics of the microstructure.

Diverse MRI techniques have been applied specifically to the study of materials for pharmaceutical and/or tissue repair applications. Investigations of the diffusive/osmotic transport mechanisms and imbibed fluid mobility have been performed via measurements of T_1/T_2 and ADC for a variety of drug delivery systems [19–21], including concurrent measurements of drug elution in polyglycolic acid materials [22]. In addition, MR has been used to directly image the matrix itself [23,24], including ^{31}P MRI of a calcium phosphate implant material (synthetic hydroxyapatite) [25].

In amorphous CPP, water molecules may diffuse freely among the chains, or they may interact with them via hydrogen bonding (which leads to gelling) or by chain scission (which produces hydroxyl end groups) [5]. In a previous work, MRI was used to acquire maps of proton density and T_1 to analyze water ingress and microstructure in non-drug-loaded CPP disks [26]. A biexponential T_1 was suggestive of water exchange between environments of relatively free and restricted mobility within the CPP microstructure, which led to hypotheses related to the effect of the microstructure on drug transport/release. However, evolution of the system between mono- and biexponential behaviors also provided evidence that the exchange frequency between these environments changes over time.

Change in a highly mobile water component is of interest, as it may provide a rate-controlling mechanism for diffusive transport of drug; further, differences in the relative proportion of free/bound water may help to explain different modes of drug release. In the present work, a concurrent elution/MRI study was conducted, and the range of MRI techniques applied to CPP bioceramics was expanded to gain better understanding of how free and bound water components evolve within the microstructure of G1 and G2 materials. It is hypothesized that G1 and G2 disks differ qualitatively in the distribution and relative proportion of the free/bound water contained within, and the study aims to interpret these results in the context of elution, to infer potential mechanisms that govern drug transport.

2. Materials and methods

2.1. CPP bioceramic samples

A raw powder of amorphous CPP was produced according to a procedure published by Pilliar et al. [3]. Degradable, antibiotic-loaded CPP disks were fabricated according to two (sequential) processes designated the "Generation 1" (G1) and "Generation 2" (G2) protocols, developed respectively by Dion et al. [7] and Petrone et al. [8] and modified here to produce smaller (4 mm diameter) disks suitable for imaging in the MRI system.

G1 disks are fabricated by first mixing CPP powder (<45 μm particle size), deionized water and vancoymycin hydrochloride (VCM) in a ratio of 150 mg CPP:60.2 μl H_2O:3.63 mg VCM. The mixture is a thick paste, which is formed into a disk shape using a polyvinylsiloxane mold. These are then subjected to a gelling step (exposure to 100% humidity at 37 °C) for 2 h, followed by a 24-h drying step. G2 disks are fabricated via an additional compaction/re-gelling step. Existing G1 disks are milled and sieved to obtain a powder (45–212 μm particle size), which is then subjected to uniaxial compaction in a die-punch at 113 MPa for 5 minutes followed by secondary gelling and drying steps.

Three G1 samples and three G2 samples were used for this experiment, and their characteristics are summarized in Table 1.

In preparation for imaging in the MRI system, each disk was mounted on a polyetherimide spacer (Ultem™) and fixed in a long, narrow glass NMR test tube (Wilmad Labglass, 7-in., 5 mm o.d.) using cyanoacrylate cement [27]. In addition to holding the samples during the MRI scans, the test tubes also contained the reservoir of elution medium. Fig. 1 illustrates the steps in sample assembly. While this geometry is more restrictive than traditional elution apparatus, it is appropriate for a biomaterial such as CPP, which is intended for implantation directly in a bone fracture.

The elution medium was a deuterium-rich phosphate buffer (0.14 molar, pH 7.2, 30% D_2O). The phosphate buffer was chosen because it is devoid of hydrogen-containing molecules (other than water) that could otherwise interfere with fluid density measurements, and the deuterium component was included to reduce probe-sample coupling (i.e. "radiation damping", which had been shown in pilot tests to impact the accuracy of the MRI relaxation measurements) [28].

Changes in disk volume due to swelling were observed via photographs of the samples acquired at each time point. Swelling was analyzed by first defining a region-of-interest (ROI) in the images, using an automated detection algorithm, then calculating the height profile of the disk and estimating the volume based on the cylindrical geometry. To define the transition point between swelling at early and later time points, a threshold method was used to determine when the swelling rate had approached sufficiently close to its asymptotic value. This was done by differentiating the volume curves using a three-point Lagrangian interpolation and by defining the threshold level from the distinctive inflection point observed in disk volume curves from preliminary tests (which also used a larger number of samples, $n = 6$).

2.2. MRI

MRI was performed on a Bruker 500 Ultrashield vertical-bore superconducting magnet (11.7 T) with an Avance 500 spectrometer. The spectrometer was controlled using XWin-NMR software (version 2.6), using a 5 mm ^{1}H probe with built-in, triple-axis (*xyz*) gradients. The probe was slightly detuned relative to the resonant frequency as a measure to further reduce probe/sample coupling [28]. Temperature in the probe was maintained at 20 °C at all times, chosen to match the ambient temperature where the samples were stored, and because the diffusion coefficient of water is a well-characterized standard at this temperature ($D = 2.0 \times 10^{-5}$ cm^2 s^{-1}) [29].

One-dimensional (1-D) axial spin–density profiles (echo time, TE = 1.547 ms; repetition time, TR = 15,900 ms) were obtained using a spin–echo imaging sequence with 35-μm resolution and an 8.98-mm field-of-view. Because of interactions between polyphosphates and metal ions, a contrast agent could not be used to

Table 1
Average characteristics of G1 and G2 samples.

Disk type	Height (mm)	Diameter (mm)	Mass (mg)	VCM loading (μg)
G1	1.7	4.0	32.7 ± 0.4	633 ± 5
G2	2.6	4.0	53.6 ± 1.3	650 ± 15