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Deterioration of the mechanical properties of calcium phosphate cements with Poly (γ-glutamic acid) and its strontium salt after *in vitro* degradation

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ABSTRACT

The mechanical reliability of calcium phosphate cements has restricted their clinical application in load-bearing locations. Although their mechanical strength can be improved using a variety of strategies, their fatigue properties are still unclear, especially after degradation. The evolutions of uniaxial compressive properties and the fatigue behavior of calcium phosphate cements incorporating poly (γ-glutamic acid) and its strontium salt after different *in vitro* degradation times were investigated in the present study. Compressive strength decreased from the 61.2 ± 5.4 MPa of the original specimen, to 51.1 ± 4.4, 42.2 ± 3.8, 36.8 ± 2.4 and 28.9 ± 3.2 MPa following degradation for one, two, three and four weeks, respectively. Fatigue life under same loading condition also decreased with increasing degradation time. The original specimens remained intact for one million cycles (run-out) under a maximum stress of 30 MPa. After degradation for one to four weeks, the specimens were able to withstand maximum stress of 20, 15, 10 and 10 MPa, respectively until run-out. Defect volume fraction within the specimens increased from 0.19 ± 0.021% of the original specimen to 0.60 ± 0.19%, 1.09 ± 0.04%, 2.68 ± 0.64% and 7.18 ± 0.34% at degradation time of one, two, three and four weeks, respectively. Therefore, we can infer that the primary cause of the deterioration of the mechanical properties was an increasing in micro defects induced by degradation, which promoted crack initiation and propagation, accelerating the final mechanical failure of the bone cement. This study provided the data required for enhancing the mechanical reliability of the calcium phosphate cements after different degradation times, which will be significant for the modification of load-bearing biodegradable bone cements to match clinical application.

1. Introduction

Calcium phosphate cements (CPCs) have been studied for more than forty years (LeGeros et al., 1982; Brown and Chow, 1985) on account of their good biocompatibility, bioactivity and osteoconductivity (Ambard and Mueninghoff, 2006). Their injectability and self-hardening ability has promoted their clinical applications, especially in minimally invasive surgical techniques such as vertebroplasty and kyphoplasty (Lewis, 2006). However, some issues, such as their relatively low strength and susceptibility to brittle fracture which restrict their application in load-bearing bone defects, still remain to be improved in CPCs (Matsumine et al., 2006; Habibovic and de Groot, 2007; The Surgeon's Committee of the Chinese Myeloma Working Group of the International Myeloma, 2016). Therefore, different blends of CPC with polymer (Mickiewicz et al., 2002; Schnieders et al., 2006; Wang et al., 2010) and other reinforcing materials (Yu et al., 2009; Canal and Ginebra, 2011) have been developed in recent studies to improve their mechanical properties. Among these modification methods, the incorporation of anionic polypeptide poly (γ-glutamic acid) (γ-PGA) (Gao et al., 2015) and the element strontium (Sr) (Baier et al., 2013; Gao et al., 2017) effectively demonstrated enhanced CPC compressive strength.

Because of cyclic physiological activities, the compressive strength of bone cements is an insufficient indicator of their mechanical reliability. Fatigue property is also important in load-bearing applications (Bohner, 2010; Sugawara et al., 2013). For non-resorbable bone cements, fatigue lifetimes have been extensively investigated with the addition of various reinforcements, methods of mixing, shapes of specimens and test conditions, *etc.* (Lewis, 2003; Ajaxon and Persson, 2014). However, the fatigue properties of CPCs are reported much less than that of the acrylic bone cements (Harmata et al., 2015).

The degradation properties of CPCs is one of the advantages that is conductive to the bone formation (Lu et al., 2002; Félix Lanao et al., 2013) and drug delivery (Ginebra et al., 2006). Meanwhile, the degradation of CPCs also leads to deterioration of mechanical properties such as the decrease of compressive strength (Hemmati et al., 2014). A

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few studies focus on the compressive strength of CPCs after degradation (Qi et al., 2008; Habraken et al., 2010), and the mechanical weakening was considered as the result of the change of the micro-structure, such as the degradation of the microsphere incorporated in CPCs. But this mechanism does not necessarily apply to all the CPCs. In addition, there is still a gap in the fatigue property after degradation of CPCs.

In this study, we investigated the effect of the *in vitro* degradation on the mechanical properties, especially the fatigue properties of Sr-γ-PGA-CPC at the macro-scale. Defects within the cement induced by the degradation were also investigated at the micro-scale to explore the mechanism of damage. Since CPCs are required to exhibit strength and fatigue resistance during bone cement degradation and new bone formation, this study provides the missing data, which is required for load-bearing modified biodegradable bone cements to be utilized for various clinical applications.

2. Material and methods

2.1. Material

Alpha-tricalcium phosphate (α-TCP) was purchased from Ensail Co., Ltd. (Beijing, China). Calcium hydrogen phosphate dihydrate ($CaHPO_4{\cdot}2H_2O$, DCPD) and disodium hydrogen phosphate (Na_2HPO_4) were purchased from Sigma Aldrich (St. Louis, USA). Poly (γ-glutamic acid) (γ-PGA, Mw = 1500–2500 kDa) was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Hydroxyapatite (HA) was synthesized in-house by the co-precipitation method reported by others (Posner and Betts, 1975; Yang et al., 2013). Tris was purchased from Sangon Biotech (Shanghai, China). All reagents were of analytical purity and used directly without further purification.

2.2. Preparation of experimental specimens

To prepare the raw CPC powders, α-TCP and DCPD at a 90:10 wt ratio, were mixed and ball-milled overnight in ethanol (CPC: agate ball: ethanol = 4: 30: 9 by mass) at 464 rpm on a planetary ball miller (PM2L, DPLIFT Machinery Co., Ltd. Shanghai, China) as previously described (Gao et al., 2017). After ball-milling, the slurry was dried at 80 °C, ground into powder and stored in a vacuum desiccator for further study. 0.25 M Na_2HPO_4 solution and γ-PGA/Sr aqueous solution were chosen as the reactive liquid phase since these materials have been reported to enhance the compressive strength (Gao et al., 2017). To prepare the γ-PGA/Sr solution, γ-PGA (H^+) was initial mixed with $SrCO_3$ at a molar ratio of -COOH: Sr^{2+} = 2:1 and then deionized (DI) water was added to prepare ~10 wt% γ-PGA/Sr aqueous solution using magnetic stirring at room temperature for approximately 4 h, finally stored at 4 °C for further study.

For preparation of the mechanical testing, the liquid and powder phases were mixed at a liquid to powder ratio of 0.45 mL/g and then packed into a cylindrical mold of 6 mm diameter and 12 mm height. After removing from the mold, the samples were incubated at 37 °C for 3 days followed by polishing with 1200-grade sandpaper to ensure the top and bottom surfaces were smooth and parallel. The polished samples were stored at room temperature for the following testing.

2.3. In vitro degradation

For the *in vitro* degradation procedure, 0.05 M Tris-HCl solution at a pH of 7.4 was prepared. The test samples were placed into centrifuge tubes and immersed in Tris-HCl solution at a temperature of 37 °C. The volume of degrading solution to test sample was greater than 20:1 and was changed every 24 h in the first week, then every week for up to 4 weeks. After immersion for one, two, three or four weeks, the samples were removed, dried at 37 °C for 2–3 days in a drying oven followed by drying at 60 °C for 3–4 days until sample weights remained stable. The weight loss was calculated using Eq. (1):

$$\text{Weight loss}\ (\%) = (M_0 - M_d)/M_0 \times 100\% \quad (1)$$

where the M_0 is the original mass, M_d is the mass after degradation for different time periods.

2.4. Quasi-static compression test

Quasi-static compression tests were performed on the original and degraded specimens to obtain their compressive moduli and mechanical strength using a mechanical testing machine (Instron E10000, USA). The samples were placed between two rigid compression platens such that the axis of the cylinder coincided with the loading axis and then tested with a displacement rate of 0.5 mm/min.

2.5. Fatigue test

Fatigue tests on the original and degraded specimens were conducted on the mechanical testing machine (Instron E10000, USA) to obtain the strain-stress hysteresis loop and the fatigue life. The samples were placed between two rigid compression platens and tested in a series of compression-compression tests with pulse cycles which was referred to the previous study (Ajaxon and Persson, 2014; Harmata et al., 2015; Ajaxon et al., 2017b). The cyclic frequency was set at 2 Hz which was clinically relevant and within the frequency range quoted in previous research on human bone (Haddock et al., 2004; Rapillard et al., 2006; Dendorfer et al., 2008). The maximum compressive stresses were chosen in the range of 5–15 MPa (ASTM, 2010) for physiological loading and 15–30 MPa for supra-physiological loading. The cyclic test mode was stress-controlling with a typical triangular wave and started from a small pre-load of 0.5 MPa. The run-out maximum fatigue life limit was set at 1 million cycles which was referred to the previous research (Harmata et al., 2015).

2.6. Weibull analysis

Weibull analysis was used to investigate the fatigue lives for each bone cement set which corresponded to each degradation time. The two-parameter Weibull distribution (Weibull, 1951; Askeland et al., 2011) was given by Eq. (2):

$$\ln\ln\left[\frac{1}{1-P(N_f)}\right] = b\ln(N_f) - b\ln(N_a) \quad (2)$$

where N_f is the cycles to failure, $P(N_f)$ is the probability of failure after N_f stress cycles, and is determined by Eq. (3) (Lewis and Janna, 2003; Weibull, 1961):

$$P(N_f) = \frac{i-3}{n+0.4} \quad (3)$$

where i is specimen rank assigned to N_f results in an ascending order, n is the total number of specimens, thus $i = 1, 2, 3, \ldots, n$. b is the Weibull modulus or shape parameter, N_a is the characteristic fatigue life or the scale parameter. From the plot of $\ln\ln\left[\frac{1}{1-P(N_f)}\right]$ *versus* $\ln(N_f)$, b and N_a are obtained from the linear fit.

2.7. Observation of microstructure

The fracture surfaces of both original and degraded specimens were observed by scanning electron microscopy (SEM, Quanta 250, FEI, 20 kV) at a magnification of 5000×. X-ray radiographs of the internal microstructure were captured by a Y. Cougar-Feinfocus X-ray imaging system (YXLON, Germany), in which spatial resolution is determined by the X-ray tube voltage and the detection efficiency. For each specimen, a series of 720 images was acquired to create three-dimensional visualization. The defects within the specimen which were induced by manufacture and degradation could be recognized and quantified.