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Fatigue performance of a high-strength, degradable calcium phosphate bone cement

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ABSTRACT

Calcium phosphate cements (CPCs) are clinically used as injectable materials to fill bone voids and to improve hardware fixation in fracture surgery. *In vivo* they are dynamically loaded; nonetheless little is known about their fatigue properties. The aim of this study was to, for the first time, investigate the fatigue performance of a high-strength, degradable (brushitic) CPC, and also evaluate the effect of cement porosity (by varying the liquid to powder ratio, L/P) and the environment (air at room temperature or in a phosphate buffered saline solution, PBS, at 37 °C) on the fatigue life. At a maximum compressive stress level of 15 MPa, the cements prepared with an L/P-ratio of 0.22 and 0.28 ml/g, corresponding to porosities of approximately 12% and 20%, had a 100% probability of survival until run-out of 5 million cycles, in air. When the maximum stress level, or the L/P-ratio, was increased, the probability of survival decreased. Testing in PBS at 37 °C led to more rapid failure of the specimens. However, the high-strength cement had a 100% probability of survival up to approximately 2.5 million cycles at a maximum compressive stress level of 10 MPa in PBS, which is substantially higher than some *in vivo* stress levels, *e.g.*, those found in the spine. At 5 MPa in PBS, all specimens survived to run-out. The results found herein are important if clinical use of the material is to increase, as characterisation of the fatigue performance of CPCs is largely lacking from the literature.

1. Introduction

Bone loss and fractures, due to, *e.g.*, osteoporosis, may call for the use of bone substituting materials. Calcium phosphate cements (CPCs) are used for this purpose as injectable materials to fill bone voids and to improve hardware fixation in fracture surgery (Larsson and Bauer, 2002; Bajammal, 2008). CPCs are self-setting and form a biomaterial that is chemically similar to the mineral content of human bone (Bohner et al., 2005; Dorozhkin, 2010), possessing biocompatible and osteoconductive properties. Some CPC compositions have shown a fast resorption rate (Apelt et al., 2004; Tamimi et al., 2012), which may be beneficial for the regrowth of new bone tissue, and some compositions have even been shown to stimulate bone tissue formation *in vivo* (Habibovic et al., 2008; Engstrand et al., 2014b, 2015).

A major drawback of CPCs is their brittleness, which limits their use in clinical applications. However, in certain cases, *e.g.*, for confined fractures and where the expected loading scenario is mainly compressive, CPCs may provide adequate support, and be the preferred choice over less biocompatible materials currently used, *e.g.*, acrylic bone cements. In order to evaluate the possible future use of CPCs in such applications, a greater understanding of the materials' fatigue properties is needed.

Mechanical characterisation of CPCs is most commonly done by quasi-static compressive loading (Tamimi et al., 2012; Zhang et al., 2014; Ajaxon and Persson, 2017), which provides a good starting point for evaluation of the material. However, the quasi-static strength alone does not provide enough information on how the material will behave in a clinical application. *In vivo*, repeated loading can be expected and hence the material's resistance to fatigue is very important in order to determine its suitability for clinical use. To aid in the prediction of the cements' behaviour *in vivo*, the fatigue properties of CPCs need to be studied. Unfortunately, the fatigue performance of CPCs alone is rarely reported in the literature: there are only a handful publications on apatite cements (Morgan et al., 1997; Jew et al., 2001; Zhao et al., 2010), one on biphasic CPC (calcium sulphate and brushite (dicalcium phosphate dihydrate)) (Harmata et al., 2015); and only one on pure acidic CPCs (brushite and monetite (dicalcium phosphate anhydrous)) (Ajaxon et al., 2017).

There is a great variation in compressive strength depending on the formulation of the CPC (Tamimi et al., 2012; Zhang et al., 2014; Ajaxon and Persson, 2017). Recent advances have led to the development of a high-strength brushite cement (Unosson and Engqvist, 2014; Engstrand

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et al., 2014a), with an average strength of 74 MPa in compression – at least twice that of human trabecular bone (Kopperdahl and Keaveny, 1998; Perilli et al., 2008), which shows that this material may be promising in certain well-defined load-bearing cases. To further evaluate this high-strength cement, the material needs to be tested under fatigue loading in order to provide more information on the material's suitability for clinical applications.

The porosity of CPCs is an important factor since it influences the degradation rate of the cements and also has a direct negative effect on the mechanical properties (Tamimi et al., 2012; Zhang et al., 2014). Several studies have investigated the influence of porosity on the compressive strength and diametral tensile strength of CPCs (Engstrand et al., 2014a; Zhang et al., 2014). However, none have reported on the influence of porosity or the largest defect (pore) size on the fatigue performance of the cements. Moreover, a wet state has previously been shown to affect the quasi-static strength of both apatite and brushite cements (Pittet and Lemaître, 2000; Gorst et al., 2006; Zhang et al., 2014; Luo et al., 2016), and therefore it can be assumed that the fatigue life of CPCs may be affected if the tests are performed in air or under physiological conditions (wet, 37 °C).

The aims of the present study were to 1) evaluate the compressive fatigue performance of a high-strength brushite cement; 2) evaluate how the fatigue performance is influenced by the porosity and the largest defect size of the cements; and 3) investigate the impact of the environment on the fatigue performance.

2. Materials and methods

2.1. Specimen preparation

All chemicals, including beta-tricalcium phosphate (ß-TCP), disodium dihydrogen pyrophosphate (SPP), citric acid, and phosphate buffered saline (PBS; containing 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4) were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA), except for monocalcium phosphate monohydrate (MCPM) which was purchased from Scharlau (Scharlau, Sentmenat, Spain).

The self-setting brushite cement was prepared by mixing MCPM (sieved to obtain particle sizes < 75 μm) and ß-TCP in a ratio of 45:55 mol% together with a citric acid solution (0.5 M) for 1 min in a mechanical mixing device (Cap Vibrator Ivoclar Vivadent AG, Schaan, Liechtenstein). SPP (1 wt%), added to the powder phase, acted as a retardant of the setting reaction (Unosson, 2014). Three different liquid to powder (L/P) ratios were used: 0.22 ml/g, 0.28 ml/g and 0.35 ml/g, to achieve cements with different porosities. The cement paste was moulded in cylindrical moulds (6 mm diameter) and specimens were left to set for 24 h in PBS at 37 °C to achieve full setting (Unosson and Engqvist, 2014). The set specimens were wet polished with SiC paper to a final height of 12 mm (specimen dimensions according to the standard ASTM F 451-08 ASTM (2008)).

2.2. Porosity measurements

The total open porosity of the wet cements was evaluated by solvent exchange, which has been previously established as a valid porosity method for wet brushite cements (Ajaxon et al., 2015). Briefly, the specimens were weighed in their wet state and the apparent volumes were determined by Archimedes' principle using a density kit (Mettler Toledo, Greifensee, Switzerland). The specimens were then immersed in isopropanol (10 ml) and left at room temperature (RT) until constant weight was achieved (24 h for L/P-ratios of 0.22 and 0.28 ml/g and 48 h for an L/P-ratio of 0.35 ml/g). Finally the weights of the specimens were recorded. The total open porosity was calculated from the ratio of the weight difference before and after isopropanol immersion and the apparent volume, taking into account the differences in density of water and solvent.

In order to investigate a possible correlation between the largest defect (pore) and the number of cycles to failure, the microstructure of all specimens was studied using micro computed tomography (micro-CT; SkyScan 1172, Bruker microCT, Kontich, Belgium) before testing them under fatigue. The scanner operated at a source voltage of 100 kV and a current of 100 μA, and the specimens were placed on top of each other in a poly(methyl methacrylate) container filled with double distilled water in order to keep them wet throughout the analysis. A Cu-Al filter was used and images were acquired using an isotropic pixel size of 13.9 μm. NRecon (Bruker microCT, Kontich, Belgium) was used to reconstruct the images. Calculations of the largest pore sizes, using a volume-equivalent sphere diameter, and total closed porosity (however, limited by the scanner resolution to pores > 13.9 μm) were performed with CTAn (Bruker, microCT, Kontich, Belgium).

2.3. Quasi-static compressive strength

The quasi-static compressive strength of the cement specimens was assessed by loading them to failure at a speed of 1 mm/min in a universal testing machine (AGS-X, Shimadzu, Kyoto, Japan) equipped with fixed compression platens and a 5 kN load cell. The specimens were kept wet until testing.

2.4. Phase characterisation

After compression testing, the cement specimens were thoroughly ground and homogenized. Six powder specimens were taken at random for analysis with X-ray diffraction (XRD), using a D8 Advance (Bruker, AXS GmbH, Karlsruhe, Germany) in a theta-theta setup with Ni-filtered Cu-Kα irradiation. Diffraction angles of 10–60° (2θ) were analysed in steps of 0.02 degrees with 0.25 s per step, while rotating the sample at a speed of 80 rpm. Rietveld refinement was applied to quantify the phase composition, using Profex (http://profex.doebelin.org) (Doebelin and Kleeberg, 2015) in combination with BGMN (http://www.bgmn.de) (Bergmann et al., 1998; Taut et al., 1998). Crystalline models were taken from PDF# 04–013-3344 (Curry and Jones, 1971) for brushite, PDF# 04–009-3755 (Dickens et al., 1971) for monetite, PDF# 04–008-8714 (Dickens et al., 1974) for β-TCP, and PDF# 04–009-3876 (Boudin et al., 1993) for beta-calcium pyrophosphate (β-CPP; a constituent of the as-received ß-TCP). No other phases were identified in the diffraction patterns. The repeatability of the quantitative phase composition was taken as 2.77 x standard deviation according to ASTM E177-14 ASTM (2013) and Döbelin (2015).

2.5. Fatigue testing

The fatigue tests were performed in two different environments. Tests in air (at RT) were performed for ease of testing and allowed the results to be compared with those of previous studies; tests in PBS at 37 °C were conducted to more closely mimic an *in vivo* situation and to evaluate the influence of the surrounding environment. All tests were performed using a dynamic materials testing system (MTS® Axial 858 Mini Bionix® II, MTS Systems Corp., Eden Prairie, MN, USA) equipped with a 5 kN load cell. An environmental testing chamber (MTS Bionix EnviroBath, MTS Systems Corp., Eden Prairie, MN, USA) with a circulating heat bath (Polystat®, Cole-Parmer, Vernon Hills, IL, USA) was connected to the materials testing system to test specimens in PBS at 37 °C.

Each specimen was subjected to a small preload of 0.5 MPa, followed by a cyclic sinusoidal constant-amplitude compression-compression load (minimum stress level of 0.5 MPa, maximum stress level as described below), at a frequency of 2 or 20 Hz. A frequency of 2 Hz was used as indicated by the standard for fatigue testing of acrylic cements for joint implant fixation ASTM F2118-03 (ASTM, 2009), as there is no standard for CPCs. A frequency of 20 Hz was used to accelerate the test. An increased frequency has previously been shown to negatively

influence the fatigue life of acrylic bone cements due to thermal softening of the material associated with a higher frequency (Ajaxon and Persson, 2014). However, it was hypothesized that a frequency increase would not influence the fatigue performance of brushite, being a ceramic cement. Initially, cements prepared with an L/P-ratio of 0.22 ml/g were tested in fatigue in air, using a maximum compressive stress level, S_{max}, of 30 MPa (based on preliminary testing). S_{max} was decreased in steps of 5 MPa until specimens survived to run-out (5 million cycles, as specified in ASTM F2118-03 ASTM (2009)). For comparison and to evaluate the influence of porosity on fatigue, L/P-ratios of 0.28 and 0.35 ml/g were tested in air at S_{max} of 15 and 30 MPa. For the fatigue tests in PBS, only the L/P ratio of 0.22 ml/g was tested and maximum stress levels of 5, 10, 15, 20 and 25 MPa were used.

Failure was taken either when a sudden decrease in load occurred (catastrophic failure) or at a specimen height reduction of 15%, as vertebral compression fractures are detected at a height reduction of 15–25% (Schwartz and Steinberg, 2005). At least 10 specimens were tested for each group. Additional fatigue data come from preliminary testing at S_{max} of 29 MPa.

2.6. Statistical analysis

IBM® SPSS® Statistics (version 22, IBM Corp., Armonk, NY, USA) was used for the statistical evaluation. A non-parametric Mann-Whitney test was used to analyse the difference in fatigue results at 2 and 20 Hz. Analysis of variance (ANOVA), using Scheffe's post-hoc test, was used to evaluate statistical differences in porosity between cements prepared with different L/P-ratios. For comparison of quasi-static compressive strengths, Welch's robust test of equality of means and Tamhane's post-hoc test were used since homogeneity of variance could not be confirmed (using Levene's test). A significance level of $\alpha = 0.05$ was used in all above tests.

The Weibull distributions were calculated using the Distribution fitting toolbox in MATLAB® (Version R2012a, The Math Works® Inc., Natick, MA, USA). Two-parameter Weibull distributions were used, and no data points were excluded.

3. Results

3.1. Porosity and quasi-static compressive strength

The total open porosities and quasi-static compressive strengths of cements prepared with different L/P-ratios are shown in Table 1. An L/P-ratio of 0.22 ml/g resulted in cements with the lowest porosity (11.2 ± 3.1%) and the highest strength (47.5 ± 12.7 MPa). By increasing the amount of liquid, an increase in porosity was seen alongside an associated decrease in strength. The highest L/P-ratio (0.35 ml/g), which resulted in a very liquid cement paste, still achieved a compressive strength of 24.1 ± 7.6 MPa with a porosity of 27.3 ± 4.2%. Significant differences in porosity were found between all L/P-ratios ($p < 0.001$), and the differences in strengths were also significant ($p < 0.003$ for all groups), while the differences in micro-CT porosity were not significant.

Table 1
Porosity (n ≥ 20 per group) and quasi-static compressive strength (n ≥ 56 per group) for cements prepared with different L/P-ratios. Standard deviations are indicated within brackets.

L/P-ratio [ml/g]	Porosity (solvent exchange) [%]	Porosity (micro-CT)[a] [%]	Quasi-static compressive strength [MPa]
0.22	11.2 (3.2)	0.5 (0.2)	47.5 (12.7)
0.28	19.7 (4.4)	0.3 (0.2)	29.1 (6.9)
0.35	27.3 (4.2)	0.6 (1.8)	24.1 (7.6)

[a] Measurement limited by the scanner resolution to pores > 13.9 μm.

3.2. Phase characterisation

The main precipitated product in the cement was brushite with a small amount of monetite. Quantitative phase composition analysis showed that the amount was about the same for all L/P-ratios (80, 79 and 78 wt% of brushite and 4, 3 and 4 wt% monetite for L/P ratios of 0.22, 0.28 and 0.35 ml/g, respectively). The analysis also revealed that the cement contained some unreacted ß-TCP (8, 8 and 9 wt% for L/P-ratios of 0.22, 0.28 and 0.35 ml/g, respectively), as well as ß-CPP (8, 9 and 10 wt% for L/P-ratios of 0.22, 0.28 and 0.35 ml/g, respectively). The repeatability was better than 1.6 wt% for all phases. XRD patterns and accuracy of the Rietveld refinement can be found in Fig. 1.

3.3. Fatigue testing

The fatigue results are summarized in Fig. 2, illustrating the relationship between maximum compressive stress, S_{max}, and number of cycles to failure, N_f, for specimens prepared with an L/P-ratio of 0.22 ml/g and tested in air (at RT) or PBS (37 °C). Initial tests performed at S_{max} of 29 MPa (included in Fig. 2) showed that no significant differences in N_f could be found between 2 and 20 Hz ($p = 0.093$), as expected. Therefore, to accelerate the testing, all subsequent tests were performed using a frequency of 20 Hz.

At S_{max} of 30 MPa, specimens tested in air generally failed rapidly (median N_f of approximately 20,000 cycles). However, by decreasing S_{max} to 25 MPa the number of cycles to failure in air increased (median N_f a factor of 100 higher compared to 30 MPa), and a few specimens (4) survived all the way to run-out (indicated in Fig. 2 with an arrow). At S_{max} of 20 MPa even more specimens tested in air (6) survived to run-out (median $N_f \approx 3\,400{,}000$ cycles), and at 15 MPa all specimens tested in air survived to run-out (10).

All specimens (10) tested in PBS at S_{max} of 5 MPa survived to run-out, and at 10 MPa all specimens except one (9) survived to run-out. At 15 MPa two specimens survived to run out (median $N_f \approx 2$ million cycles), whereas at 20 and 25 MPa specimens failed more rapidly (no specimens survived to run-out at either stress level, median $N_f \approx$ 80,000 cycles at 20 MPa and median $N_f \approx$ 6500 cycles at 25 MPa).

The relationship between number of cycles to failure and L/P-ratio can be seen in Fig. 3. At the lowest S_{max} in air (15 MPa) all specimens prepared with L/P-ratios of 0.22 and 0. 28 ml/g (average porosities of 11.2% and 19.7%, respectively) survived to run-out. However, for the highest L/P-ratio (0.35 ml/g, average porosity of 27.3%) the variation in fatigue life was large. At S_{max} of 30 MPa, a decrease in fatigue life with an increase in L/P-ratio (and porosity) could be discerned. Comparing the fatigue life of specimens tested in PBS and in air, at S_{max} of 15 MPa the specimens survived longer when tested in air compared to PBS. However, at the lowest S_{max} in PBS (5 MPa) all specimens survived to run-out.

The probability of survival for cements prepared with different L/P-ratios and tested in air at different S_{max} can be seen in Fig. 4a. At S_{max} of 15 MPa, the cements prepared with an L/P-ratio of 0.22 and 0.28 ml/g had a 100% probability of survival, and as the maximum stress level was increased the probability of survival decreased. At the same S_{max}, the probability of survival decreased when the L/P-ratio was increased to 0.35 ml/g. At S_{max} of 30 MPa, the probability of survival was low for all L/P-ratios.

Fig. 4b shows the probability of survival when fatigue tests were performed in PBS. The specimens were prepared with an L/P-ratio of 0.22 ml/g. All specimens survived to run-out when S_{max} was set to 5 MPa and all specimens, except one, survived to run-out when S_{max} was set to 10 MPa. By increasing S_{max} to 15, 20 and 25 MPa, the probability of survival decreased dramatically.

No correlation could be found between either largest pore size or total closed porosity determined by micro-CT and number of cycles to failure (data can be found in supplementary information).

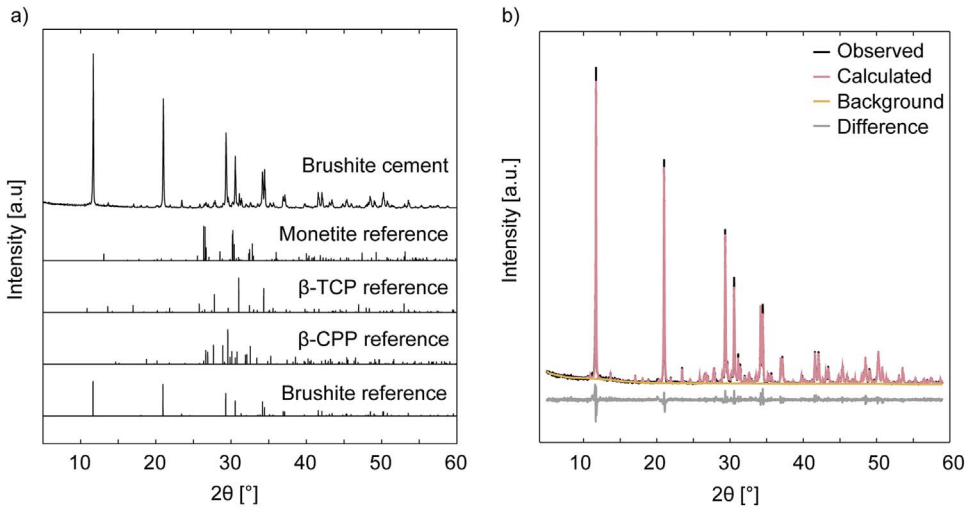

Fig. 1. a) Representative XRD pattern of the brushite cement (with an L/P-ratio of 0.22 ml/g) shown together with references of the identified phases, and b) representative pattern showing the accuracy of the Rietveld refinement.

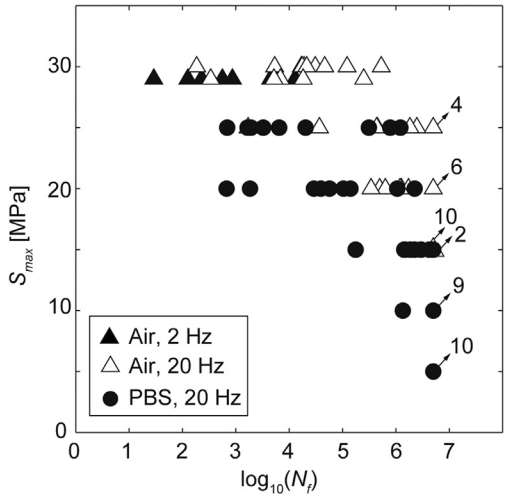

Fig. 2. Number of cycles to failure, N_f, at different maximum stress levels, S_{max} for specimens tested in air (at 2 and 20 Hz) and in PBS (at 20 Hz). All specimens were prepared with an L/P-ratio of 0.22 ml/g. Run-out is marked with an arrow and the number of specimens that survived to this limit is indicated (clarification: *e.g.*, at S_{max} of 15 MPa, 10 specimens in air and 2 specimens in PBS survived to run-out).

4. Discussion

This study presented the compressive fatigue performance of a high-strength brushite cement, and investigated how cement porosity and environment influence the fatigue life of the cements.

The phase composition of the brushite cement investigated in this study was similar to that which had been previously found for the same cement formulation (Unosson and Engqvist, 2014), even though the amount of unreacted ß-TCP and ß-CPP was slightly different (approximately 8 wt% ß-TCP and 9 wt% ß-CPP herein, compared to 11 wt % ß-TCP and 6 wt% ß-CPP in the study by Unosson and Engqvist). There are also differences in the quasi-static compressive strength of the brushite cements between the studies, which, besides the variation in phase composition may be explained by differences in particle size distribution of reactant powders (different batches of MCPM and ß-TCP were used in the two publications), as the amount of ß-TCP (added as a filler material it has a positive effect on the strength up to a certain amount) and particle size (smaller particle sizes lead to a CPC with a higher strength) have previously been shown to have an effect on the strength (Unosson, 2014).

The fatigue life of the high-strength brushite cements tested in air at S_{max} of 15 MPa was found to be higher (run-out for 0.22 and 0.28 ml/g), compared to that which had been found before for brushite cements tested in air (N_f ranging between a few hundred cycles up to 1000 at a stress level of 13 MPa) (Ajaxon et al., 2017). Even specimens prepared with the highest L/P-ratio herein (0.35 ml/g, median $N_f \approx$ 20,000 cycles), corresponding to the cements with the lowest quasi-static strength (~ 24 MPa) and the highest porosity (~ 27%), survived longer compared to the cements in the above-mentioned study. This was not surprising, since the wet quasi-static compressive strengths at all L/P-ratios presented here were also much higher compared to the strength of the moist brushite cements previously evaluated (14.0 ± 3.5 MPa) (Ajaxon et al., 2017). Moreover, for these previously evaluated acidic CPCs, the strength of dry cements containing up to 23 wt% monetite (29.4 ± 8.0 MPa) is similar to that of wet cements prepared with an L/P-ratio of 0.28 ml/g in the present study (29.1 ± 6.9 MPa). Simultaneously, the resistance to fatigue is higher for the brushite cements presented here (in the previous publication N_f ranged between 800,000–5 million at S_{max} of 13 MPa (Ajaxon et al., 2017)). The differences in fatigue life (as well as quasi-static strength) between the high-strength brushite cement evaluated herein and the previously studied brushite cement formulation are likely due to differences in pore content and distribution as well as crystal entanglement, which will affect the mechanical properties, and in particular the propagation of cracks within the material.

A clear effect related to the environment could be discerned, the probability of survival decreased when specimens were tested in PBS compared to in air at the same stress level. A parallel can be drawn between fatigue tests in air or in PBS to differences seen in wet and dry quasi-static strengths of CPCs (Luo et al., 2016), as well as for a self-setting calcium sulphate-based cement (Koh et al., 2014): the strength is generally lower for a wet cement compared to a dry cement, and a lower applied stress level is required for the specimens to survive to run-out in PBS compared to air. A likely explanation for this behaviour has previously been proposed by Andrews (Andrews, 1946): in a cement where the mechanical rigidity of the structure is provided by entanglement of crystals, it is possible that fracture of the structure could occur when the frictional force between interlocked crystals is exceeded, rather than breakage of individual crystals. When water is introduced in the structure, the frictional force is reduced, which means that a crack will propagate easier than if water were not present. Other factors that could be hypothesized to contribute are the incompressibility of water and, for fatigue, degradation of the cement in an aqueous environment. This last point is unlikely to have a large effect in the present study due to the relatively short tests performed (run-out corresponded to approximately 3 days of testing at a frequency of 20 Hz).

While differences in fatigue life could be found between cement

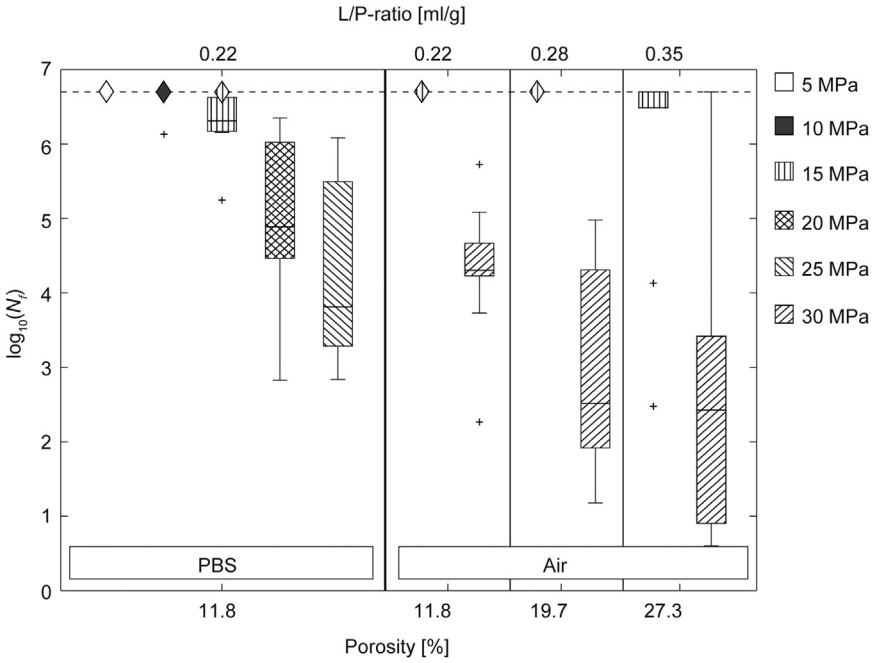

Fig. 3. Boxplot of number of cycles to failure, N_f, in air and PBS at different S_{max} for specimens prepared with different L/P-ratios. Specimens that survived to run-out are marked with a diamond.

batches of different porosity, as induced by different L/P ratios, no correlation could be found for a specific L/P ratio between porosity or largest pore and fatigue life (supplementary information). It should be noted that the total porosity as determined by micro-CT only took into account pores larger than 13.9 μm, hence excluding most pores in the cement (Engstrand Unosson et al., 2015). However, the solvent exchange method has been found to capture the total porosity well (Ajaxon et al., 2015), but no correlation could be discerned for that either. The lack of correlation between total porosity or largest pore and the fatigue life within a batch could be due to the limited amount of samples tested, as well as the chosen stress levels: it is likely that an individual critical defect will have the largest influence at stress levels in between the plateaus of the S-N curve. Therefore, due to the large spread in porosity and largest pore size within a batch, a very large number of samples may be required at intermediate stress levels in order to establish correlations.

The results from the fatigue tests performed in PBS show that the fatigue life of the investigated high-strength brushite cement is greater compared to a previously studied biphasic cement in compressive fatigue, which had a median fatigue life of 23 500 cycles at 5 MPa, 236 cycles at 10 MPa, and 4 cycles at 15 MPa) (Harmata et al., 2015). Differences in, *e.g.*, crystal entanglement, porosity and size distribution of pores of different cement compositions may explain their different behaviour in fatigue. The other previously published fatigue studies of CPCs are not directly comparable to the results presented herein, as they evaluated fatigue under 3-point bending (Zhao et al., 2010) or the fatigue crack growth rate (Morgan et al., 1997; Jew et al., 2001).

The fatigue life of the investigated high-strength brushite cement in PBS at 5 MPa is higher than that of human trabecular bone (between 200,000–440,000 cycles at S_{max} of approximately 2–3 MPa, and run-out limit taken as at least one week of testing using a maximum frequency of approximately 3 Hz) (Haddock et al., 2004). A living bone would normally repair micro-cracks, which means this comparison is not fully representative. However, compared to compressive loads measured *in vivo* in the spine (0.1–2.3 MPa) (Wilke et al., 1999) the experimental cements studied herein had a 100% probability of survival at a compressive stress level twice as high as the *in vivo* measured loads.

The most important limitation to this study relates to the applicability of the fatigue results of the brushite cements to the *in vivo* situation. The used frequency is higher than what can be expected *in vivo*, and therefore tests were shorter and any effect of degradation was not taken into account. If future studies were to include longer tests, the effect of degradation should be taken into consideration. Moreover, any effects the frequency has on the fatigue results performed in PBS should

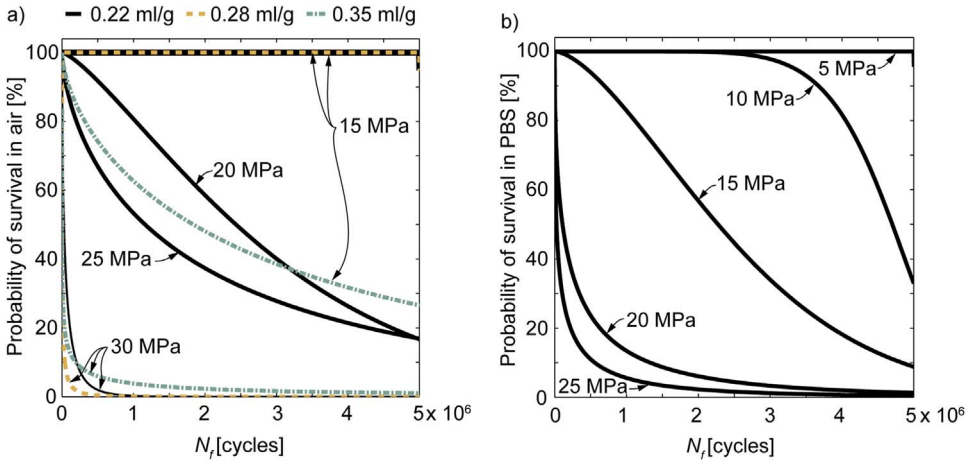

Fig. 4. Probability of survival at different S_{max} for specimens tested a) in air and b) in PBS. In a) the L/P-ratio varied, whereas in b) all specimens were prepared with an L/P-ratio of 0.22 ml/g.

be investigated. At present it has only been shown that there was no significant difference between the two frequencies used when the tests were performed in air. Still, the results found herein are important if clinical use of this type of material is to increase, as characterisation of the fatigue performance of CPCs are largely lacking from the literature. In fact, this is the first time these properties have been reported for a high-strength CPC. Another limitation is that the number of specimens tested in fatigue is lower than that which the standard proposes (15) (ASTM, 2009). On the other hand, more stress levels were tested than are specified in the standard (3). Moreover, ceramic cements have been suggested for use in vertebroplasty (Blattert et al., 2009; Lewis, 2006; Lewis et al., 2008), and the chosen stress levels are higher than the suggested value for fatigue testing of materials for spinal applications (5 MPa) (Persson and Berg, 2013). Furthermore, the tested stress levels are in the range of reported compressive quasi-static strengths of human trabecular bone (1–30 MPa) (Kopperdahl and Keaveny, 1998; Perilli et al., 2008), and considerably higher compared to those used for fatigue testing of human vertebral trabecular bone (0.2–5 MPa) (Haddock et al., 2004).

As large differences in probability of survival were seen between cements with different porosities when the fatigue tests were performed in air, future studies should investigate the effect of porosity on the fatigue performance when tests are performed in PBS. Moreover, future studies should also include other formulations of CPCs, as it is likely that not only differences in porosity, but also differences in composition and microstructure will have an effect on the fatigue performance.

5. Conclusions

The compressive fatigue properties of a high-strength brushite cement were evaluated, and the effect of porosity and environment on these properties were assessed. The fatigue life was seen to decrease with an increase in porosity (in air), as expected. For all L/P-ratios investigated (in air), the probability of survival was low when a maximum compressive stress of 30 MPa was used, whereas at 15 MPa a 100% probability of survival was found for specimens with average porosities of 12–20% (L/P-ratios of 0.22 and 0.28 ml/g). The environment was seen to affect the fatigue life: specimens failed more rapidly in PBS compared to air at the same stress level. However, in PBS the high-strength brushite cement investigated had a 100% probability of survival when a maximum compressive stress level of 5 MPa was used. This stress level is in the range of the quasi-static compressive strength of human trabecular bone, and twice as high compared to loads measured in the spine *in vivo*, which suggests that this material may be a suitable candidate for certain applications where mainly compressive loads are expected. This study demonstrates the importance of performing fatigue testing of CPCs, as the cements can fail catastrophically under stresses that are considerably lower than the quasi-static strength of the material (at least 30% of the quasi-static strength). A greater knowledge of the fatigue performance of degradable calcium phosphate cements is crucial to increase their clinical use.

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Conflicts of interest

There are no conflicts to declare.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jmbbm.2017.12.005.

References

Ajaxon, I., Maazouz, Y., Ginebra, M.P., Öhman, C., Persson, C., 2015. Evaluation of a porosity measurement method for wet calcium phosphate cements. J. Biomater. Appl. 30, 526–536. http://dx.doi.org/10.1177/0885328215594293.

Ajaxon, I., Mägi, C.Ö., Persson, C., 2017a. Compressive fatigue properties of an acidic calcium phosphate cement. J. Mater. Sci: Mater. Med. 28, 1–7. http://dx.doi.org/10.1007/s10856-017-5851-5.

Ajaxon, I., Persson, C., 2017b. Mechanical properties of brushite calcium phosphate cements. In: Shi, D. (Ed.), The World Scientific Encyclopedia of Nanomedicine and Bioengineering II: Bioimplants, Regenerative Medicine, and Nano-Cancer Diagnosis and Phototherapy - Volume 3: Design of Bioactive Materials for Bone Repair and Regeneration. World Scientific Pte Ltd., Singapore, pp. 285–300.

Ajaxon, I., Persson, C., 2014. Compressive fatigue properties of a commercially available acrylic bone cement for vertebroplasty. Biomech. Model Mechanobiol. 13, 1199–1207. http://dx.doi.org/10.1007/s10237-014-0566-8.

Andrews, H., 1946. The effect of water contents on the strength of calcium sulphate plaster products. J. Soc. Chem. Ind.: Trans. Commun. 125–128.

Apelt, D., Theiss, F., El-Warrak, A.O., Zlinszky, K., Bettschart-Wolfisberger, R., Bohner, M., Matter, S., Auer, J.A., Rechenberg, von, B., 2004. In vivo behavior of three different injectable hydraulic calcium phosphate cements. Biomaterials 25, 1439–1451. http://dx.doi.org/10.1016/j.biomaterials.2003.08.073.

American Society for Testing and Materials, 2008. ASTM F 451-08: Standard Specification for Acrylic Bone Cement. ASTM, West Conshohocken, PA.

American Society for Testing and Materials, 2013. ASTM E177-14: Standard Practise for Use of the Terms Precision and Bas in ASTM Test Methods. ASTM, West Conshohocken, PA.

American Society for Testing and Materials, 2009. ASTM F2118-03: Standard Test Method for Constant Amplitude of Force Controlled Fatigue Testing of Acrylic Bone Cement Materials.

Bajammal, S.S., 2008. The use of calcium phosphate bone cement in fracture treatment a meta-analysis of randomized trials. J. Bone Jt. Surg. Am. 90, 1186–1196. http://dx.doi.org/10.2106/JBJS.G.00241.

Bergmann, J., Friedel, P., Kleeberg, R., 1998. BGMN - a new fundamental parameters based Rietveld program for laboratory X-ray sources, it's use in quantitative analysis and structure investigations. IUCr Comm. Powder Diffr. Newsl. no. 20, 5–8.

Blattert, T.R., Jestaedt, L., Weckbach, A., 2009. Suitability of a calcium phosphate cement in osteoporotic vertebral body fracture augmentation: a controlled, randomized, clinical trial of balloon kyphoplasty comparing calcium phosphate versus polymethylmethacrylate. Spine 34, 108–114. http://dx.doi.org/10.1097/BRS.0b013e31818f8bc1.

Bohner, M., Gbureck, U., Barralet, J.E., 2005. Technological issues for the development of more efficient calcium phosphate bone cements: a critical assessment. Biomaterials 26, 6423–6429. http://dx.doi.org/10.1016/j.biomaterials.2005.03.049.

Boudin, S., Grandin, A., Borel, M.M., Leclaire, A., Raveau, B., 1993. Redetermination of the β-Ca2P2O7 structure. Acta Crystallogr C C49, 2062–2064. http://dx.doi.org/10.1107/S0108270193005608.

Curry, N.A., Jones, D.W., 1971. Crystal structure of brushite, calcium hydrogen orthophosphate dihydrate: a neutron-diffraction investigation. J. Chem. Soc. A 3725–3729. http://dx.doi.org/10.1039/j19710003725.

Dickens, B., Bowen, J.S., Brown, W.E., 1971. A refinement of the crystal structure of CaHPO4 (synthetic monetite). Acta Crystallogr C B28, 797–806. http://dx.doi.org/10.1107/S056774087200322X.

Dickens, B., Schroeder, L.W., Brown, W.E., 1974. Crystallographic studies of the role of Mg as a stabilizing impurity in β-Ca3(PO4)2. The crystal structure of pure β-Ca3(PO4)2. J. Solid State Chem. 10, 232–248.

Doebelin, N., Kleeberg, R., 2015. Profex: a graphical user interface for the Rietveld refinement program BGMN. J. Appl. Cryst. 48, 1573–1580. http://dx.doi.org/10.1107/S1600576715014685.

Dorozhkin, S.V., 2010. Bioceramics of calcium orthophosphates. Biomaterials 31, 1465–1485. http://dx.doi.org/10.1016/j.biomaterials.2009.11.050.

Döbelin, N., 2015. Interlaboratory study on the quantification of calcium phosphate phases by Rietveld refinement. Powder Diffr. 30, 231–241. http://dx.doi.org/10.1017/S088571561500038X.

Engstrand, J., Persson, C., Engqvist, H., 2014a. The effect of composition on mechanical properties of brushite cements. J. Mech. Behav. Biomed. 29, 81–90. http://dx.doi.org/10.1016/j.jmbbm.2013.08.024.

Engstrand, T., Kihlström, L., Neovius, E., Skogh, A.-C.D., Lundgren, T.K., Jacobsson, H., Bohlin, J., Åberg, J., Engqvist, H., 2014b. Development of a bioactive implant for repair and potential healing of cranial defects. J. Neurosurg. 120, 273–277. http://dx.doi.org/10.3171/2013.6.JNS1360.

Engstrand, T., Kihlström, L., Lundgren, K., Trobos, M., Engqvist, H., Thomsen, P., 2015. Bioceramic Implant Induces Bone Healing of Cranial Defects. Plast. Reconstr. Surg. Glob. Open 3, e491. http://dx.doi.org/10.1097/GOX.0000000000000467.

Engstrand Unosson, J., Persson, C., Engqvist, H., 2015. An evaluation of methods to determine the porosity of calcium phosphate cements. J Biomed Mater Res B 103. pp. 62–71. http://dx.doi.org/10.1002/jbm.b.33173.

Gorst, N.J.S., Perrie, Y., Gbureck, U., Hutton, A.L., Hofmann, M.P., Grover, L.M., Barralet, J.E., 2006. Effects of fibre reinforcement on the mechanical properties of brushite cement. Acta Biomater. 2, 95–102. http://dx.doi.org/10.1016/j.actbio.2005.09.001.

Habibovic, P., Gbureck, U., Doillon, C., Bassett, D.C., van Blitterswijk, C.A., Barralet, J.E., 2008. Osteoconduction and osteoinduction of low-temperature 3D printed bioceramic implants. Biomaterials 29, 944–953. http://dx.doi.org/10.1016/j.biomaterials.2007.10.023.

Haddock, S.M., Yeh, O.C., Mummaneni, P.V., Rosenberg, W.S., Keaveny, T.M., 2004.

Similarity in the fatigue behavior of trabecular bone across site and species. J. Biomech. 37, 181–187. http://dx.doi.org/10.1016/S0021-9290(03)00245-8.
Harmata, A.J., Uppuganti, S., Granke, M., Guelcher, S.A., Nyman, J.S., 2015. Compressive fatigue and fracture toughness behavior of injectable, settable bone cements. J. Mech. Behav. Biomed. Mater. 51, 345–355. http://dx.doi.org/10.1016/j.jmbbm.2015.07.027.
Jew, V.C., Morgan, J.P., Dauskardt, R.H., 2001. Strength, toughness and fatigue of an apatite cement, In: Proceedings of Presented at the Materials Research Society Symposium, Biomaterials for Drug Delivery and Tissue …, pp. LL3.5.1–LL3.5.6.
Koh, I., López, A., Helgason, B., Ferguson, S.J., 2014. The compressive modulus and strength of saturated calcium sulphate dihydrate cements: implications for testing standards. J. Mech. Behav. Biomed. 34, 187–198.
Kopperdahl, D.L., Keaveny, T.M., 1998. Yield strain behavior of trabecular bone. J. Biomech. 31, 601–608.
Larsson, S., Bauer, T.W., 2002. Use of injectable calcium phosphate cement for fracture fixation: a review. Clin. Orthop. Relat. Res. 395, 23–32.
Lewis, G., 2006. Injectable bone cements for use in vertebroplasty and kyphoplasty: state-of-the-art review. J. Biomed. Mater. Res. B 76B, 456–468. http://dx.doi.org/10.1002/jbm.b.30398.
Lewis, G., Schwardt, J.D., Slater, T.A., Janna, S., 2008. Evaluation of a synthetic vertebral body augmentation model for rapid and reliable cyclic compression life testing of materials for balloon kyphoplasty. J. Biomed. Mater. Res. B 87B, 179–188. http://dx.doi.org/10.1002/jbm.b.31089.
Luo, J., Ajaxon, I., Ginebra, M.-P., Engqvist, H., Persson, C., 2016. Compressive, diametral tensile and biaxial f lexural strength of cutting-edge calcium phosphate cements. J. Mech. Behav. Biomed. Mater. 60, 617–627. http://dx.doi.org/10.1016/j.jmbbm.2016.03.028.
Morgan, E., Yetkinler, D., Constantz, B., Dauskardt, R., 1997. Mechanical properties of carbonated apatite bone mineral substitute: strength, fracture and fatigue behaviour. J. Mater. Sci: Mater. Med. 8, 559–570.
Perilli, E., Baleani, M., Öhman, C., Fognani, R., Baruffaldi, F., Viceconti, M., 2008. Dependence of mechanical compressive strength on local variations in micro-architecture in cancellous bone of proximal human femur. J. Biomech. 41, 438–446. http://dx.doi.org/10.1016/j.jbiomech.2007.08.003.
Persson, C., Berg, S., 2013. Strategies towards injectable, load-bearing materials for the intervertebral disc: a review and outlook. J. Mater. Sci: Mater. Med. 1, 1–10. http://dx.doi.org/10.1007/s10856-012-4776-2.
Pittet, C., Lemaître, J., 2000. Mechanical characterization of brushite cements: a Mohr circles' approach. J. Biomed. Mater. Res. 53, 769–780.
Schwartz, E.N., Steinberg, D., 2005. Detection of vertebral fractures. Curr. Osteoporos. Rep. 3, 126–135.
Tamimi, F., Sheikh, Z., Barralet, J., 2012. Dicalcium phosphate cements: brushite and monetite. Acta Biomater. 8, 474–487. http://dx.doi.org/10.1016/j.actbio.2011.08.005.
Taut, T., Kleeberg, R., Bergmann, J., 1998. Seifert software: the new Seifert Rietveld program BGMN and its application to quantitative phase analysis. Mater. Struct. 5, 57–66.
Unosson, J., 2014. Physical Properties of Acidic Calcium Phosphate Cements. Uppsala University, Uppsala.
Unosson, J., Engqvist, H., 2014. Development of a resorbable calcium phosphate cement with load bearing capacity. Bioceram. Dev. Appl. 4, 074. http://dx.doi.org/10.4172/2090-5025.1000074.
Wilke, H.J., Neef, P., Caimi, M., Hoogland, T., Claes, L.E., 1999. New in vivo measurements of pressures in the intervertebral disc in daily life. Spine 24, 755.
Zhang, J., Liu, W., Schnitzler, V., Tancret, F., Bouler, J.-M., 2014. Calcium phosphate cements for bone substitution: chemistry, handling and mechanical properties. Acta Biomater. 10, 1035–1049. http://dx.doi.org/10.1016/j.actbio.2013.11.001.
Zhao, L., Burguera, E.F., Xu, H.H.K., Amin, N., Ryou, H., Arola, D.D., 2010. Fatigue and human umbilical cord stem cell seeding characteristics of calcium-phosphate-chitosan-biodegradable fiber scaffolds. Biomaterials 31, 840–847. http://dx.doi.org/10.1016/j.biomaterials.2009.09.106.