

Injectability and mechanical properties of magnesium phosphate cements

Claus Moseke · Vasileios Saratsis · Uwe Gbureck

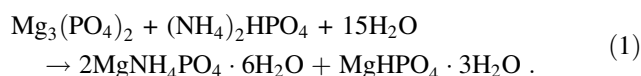
Received: 29 June 2011 / Accepted: 29 August 2011 / Published online: 14 September 2011
© Springer Science+Business Media, LLC 2011

Abstract Up to now magnesium phosphate cements are mainly being utilized in wastewater treatment due to their adsorptive properties. Recently they also have been shown to have a high potential as degradable biocements for application as replacement materials for bone defects. In comparison to degradable calcium phosphate cements they have the advantage of setting at neutral pH, which is favorable in biological environment. In this study two parameters of the cement composition, namely powder-to-liquid ratio (PLR) and citrate content, were varied in order to optimize the injectability properties of the cement paste and the mechanical properties of the reaction product. These properties were determined by means of testing setting time and temperature, paste viscosity, and injectability as well as phase composition and compressive strength of the set cements. Best results were obtained, when the cements were prepared with a PLR of 2.5 and a binder liquid consisting of an aqueous solution of 3 mol/l diammonium hydrogen phosphate and 0.5 mol/l diammonium citrate.

1 Introduction

Major clinical demands for artificial bone substitutes are firstly their ability to degrade in vivo with subsequent formation of new hard tissue and secondly the possibility to inject the material into the defect for minimally invasive surgical techniques. Common degradable materials in

clinical use are for example ceramics based on tricalcium phosphate [1] or self setting cements which form dicalcium phosphate dihydrate (brushite) [2]. The latter has the advantage that a mechanically stable implant is formed in situ at the application site, which has been shown to degrade over a time period of 3–6 months [3–5]. Major concerns about a broad clinical use of brushite cements are their relatively poor mechanical properties compared to apatite cements and their strong acidic pH value during setting which can lead to the release of acid into the surrounding tissue around the implant [6]. An interesting alternative bio cement is based upon the formation of magnesium ammonium phosphate hexahydrate (struvite, $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) from farringtonite [$\text{Mg}_3(\text{PO}_4)_2$] and diammonium hydrogen phosphate [$(\text{NH}_4)_2\text{HPO}_4$] due to the following reaction equation:



Struvite is a natural mineral which occurs as pathological calcification in kidney stones [7]. This kind of cement has the advantage of maintaining a neutral pH value during setting, since both the cement powder, consisting of MgHPO_4 and $\text{Mg}_3(\text{PO}_4)_2$, as well as the cement liquid (an aqueous solution of $(\text{NH}_4)_2\text{HPO}_4$) are non-acidic compounds. Struvite cements are thought to be chemically degradable in vivo similar to brushite due to the relatively high solubility with a solubility product in the range of 5.21×10^{-15} [$\text{pK}(\text{sp}) = 14.28$] to 2.12×10^{-13} [$\text{pK}(\text{sp}) = 12.67$] over a pH range of 7.01–9.62 [8]. Bhuiyan et al. found a maximum solubility of struvite in deionized water at 35°C of 212.7 (± 3.8) mg [9]. Few studies have investigated struvite forming cements for biomedical applications, which showed high strength of the

C. Moseke (✉) · V. Saratsis · U. Gbureck
Department of Functional Materials in Medicine and Dentistry,
School of Dentistry, University of Würzburg, Pleicherwall 2,
97070 Würzburg, Germany
e-mail: claus.moseke@fmz.uni-wuerzburg.de

materials (compressive strength >50 MPa) and an appropriate setting time of 3–10 min which meets clinical demands [10–13].

The rheological properties of calcium phosphate cement pastes are, similarly to fresh Portland cement [14], determined by the particle size and distribution of the components, the powder-to-liquid ratio (PLR), and the surface charge of the particles in contact with the liquid phase. A liquefying effect for calcium phosphate cement pastes is obtained by efficient dispersion of particle agglomerates due to electrostatic repulsion of the particles. Dispersion can be achieved by modification of the liquid phase with trisodium citrate, which adsorbs onto surfaces of both cement components and reaction products leading to a high zeta-potential and hence repulsion of the particles [15].

The current study deals with the injectability properties of struvite forming cements, which set by reaction of trimagnesium phosphate ($\text{Mg}_3(\text{PO}_4)_2$, farringtonite) and highly concentrated ammonium phosphate solution. Of particular interest was the question, how the cement viscosity could be reduced to obtain an adequate injectability of the product. Influential parameters to be determined were the variation of PLR and the partial replacement of diammonium hydrogen phosphate by diammonium citrate in order to achieve particle repulsion by the build-up of surface charges. The influence of the varied parameters was determined by testing compressive strength, setting time, reaction temperature, phase composition, and injectability of the different cement compositions.

2 Materials and methods

2.1 Cement fabrication

Farringtonite [$\text{Mg}_3(\text{PO}_4)_2$] was synthesized by heating a mixture of newberyite ($\text{MgHPO}_4 \cdot 3\text{H}_2\text{O}$; Sigma-Aldrich, Steinheim, Germany) and magnesium hydroxide [$\text{Mg}(\text{OH})_2$; Sigma-Aldrich, Steinheim, Germany] to 1,000°C for 5 h followed by quenching to room temperature in a desiccator. The product consisted of phase-pure and highly crystalline farringtonite as verified by X-ray diffraction analysis (XRD). The sintered cake was crushed with a pestle and mortar and passed through a 355 μm sieve. Milling of $\text{Mg}_3(\text{PO}_4)_2$ was performed in a planetary ball mill (PM400 Retsch, Germany) at 200 rpm with 500 ml agate jars, 4 agate balls (30 mm) and a load of 125 g $\text{Mg}_3(\text{PO}_4)_2$ per jar for 2 h.

Cement pastes were produced by mixing 3 g $\text{Mg}_3(\text{PO}_4)_2$ powder and diammonium hydrogen phosphate [$(\text{NH}_4)_2\text{HPO}_4$] solutions on a glass slab for 30 s at PLRs between 1.0 and 3.3 g/ml. Cement liquids were aqueous solutions of either pure $(\text{NH}_4)_2\text{HPO}_4$ or mixtures of $(\text{NH}_4)_2\text{HPO}_4$ and

diammonium citrate [$(\text{NH}_4)_2\text{C}_6\text{H}_6\text{O}_7$] with a total salt concentration of 3.5 mol/l. In order to prepare samples for compressive strength testing the cement paste was filled in cuboid silicon rubber moulds (12 × 6 × 6 mm) and allowed to set at 37°C for 24 h in a water bath. A cuboid-shaped geometry was used since it enabled to prepare high quality samples without macro-defects and precompaction of the cement paste, an undesired effect usually occurring with the preparation of cylindrical samples. Wet samples ($n = 6$ per condition) with an aspect ratio of 2:1 were tested in axial compression at a crosshead speed of 1 mm/min using a static mechanical testing machine Zwick 1440 (Zwick, Ulm, Germany) and a 5 kN load cell. The density of the samples was calculated from the measured weight and dimensions. The initial setting time of the cement was measured in a humidity chamber at 37°C and >90 % humidity using the Gilmore needle test with a needle of 113.98 g and 2.117 mm diameter according to ASTM 266 standard. For statistical analysis the Anova t-test of Excel was performed. In addition, paste moldability was tested and evaluated under normal ambient conditions (21°C and ca. 40 % humidity).

2.2 Injectability

The injectability was measured by mixing 10 g of cement powder with the appropriate amount of liquid on a glass slab. The cement paste was transferred into a 10 ml syringe by means of a spatula. The paste was extruded through a 1.1 × 30 mm needle (19 G × 1/4") with an internal diameter of 800 μm using the pre-mentioned mechanical testing machine at a cross-head speed of 20 mm/min and a maximum load of 300 N. The mass m_{injected} of the paste injected through the needle and the mass m_{initial} of cement initially contained in the syringe were determined and the injectability (I) was calculated according to:

$$I = \frac{m_{\text{injected}}}{m_{\text{initial}}} \cdot 100 \% \quad (2)$$

2.3 X-ray diffraction analysis (XRD) and scanning electron microscopy (SEM)

X-ray diffraction patterns of set cements were recorded on a diffractometer D5005 (Siemens, Karlsruhe, Germany), using Cu-K_α radiation with a voltage of 40 kV and a tube current of 50 mA. Diffraction data were collected in a 2 θ range from 20 to 40° with a step size of 0.02° and a normalized count time of 1 s/step. The phase composition was checked by means of JCPDS reference patterns for farringtonite (PDF-Ref. 033–0876) and struvite (PDF-Ref. 003–0240). Scanning electron micrographs of the set cements were taken with a DSM940 (Zeiss, Oberkochen,

Germany). The cylindrical cement samples were broken apart and the fracture surface was gold-coated to avoid build-up of surface charges.

2.4 Thermal measurements

The temperature increase during the setting reaction was measured with a thermocouple (Votcraft Data-Logger K202, Conrad Electronics, Germany) which was placed in the middle of a cement paste formed from 10 g cement powder at a PLR of 2.0. These measurements were performed at 37°C, whereas the paste was placed in a polystyrene mould for temperature insulation.

2.5 Zeta potential measurements

The effective surface charges of the farringtonite and struvite particles in contact with an aqueous electrolyte were determined from zeta-potential measurements, which were performed on a Zeta-Sizer 3000 (Malvern Instruments, Herrenberg, Germany) in double distilled water and three different electrolytes [0.05 M $(\text{NH}_4)_2\text{HPO}_4$, 0.05 M $(\text{NH}_4)_2\text{citrate}$, and 0.03 M $(\text{NH}_4)_2\text{HPO}_4$ + 0.02 M $(\text{NH}_4)_2\text{citrate}$]. Measurements were performed 10 times for each electrolyte composition and the average potential and the standard deviation were calculated.

3 Results

Mechanical testing of magnesium phosphate cements depending on the powder to liquid ratio (Table 1) showed compressive strengths of 40–55 MPa for a broad PLR range between 1.5 and 3.3 g/ml. A decrease of the mechanical performance to 20 MPa was observed at lower PLR of 1.0 g/ml, with a simultaneous decrease of the

cement density from approximately 1.9 g/ml at PLR 3.3–1.58 g/ml at PLR 1.0 g/ml. The Cement pastes could be molded easily at high PLR of 3.0–3.3 g/ml with setting times obtained by the Gilmore needle test of about 2 min. Lower PLR led to a decrease of the initial viscosity and the cements became paste-like after 2–23 min with setting times of 6–16 min.

Figure 1 shows the diffraction patterns obtained from XRD measurements of cements set with PLR 1.5, 2.5, and 3.3. All patterns show the typical peaks of struvite and farringtonite according to the PDF data base and hence indicate the conversion of farringtonite to struvite during the setting reaction. However, the increasing struvite peaks with decreasing PLR show that more farringtonite reacted to struvite at lower PLR. SEM micrographs of the fracture surfaces (not shown here) revealed different crystal morphologies for different PLRs. At PLR 1.0 the crystals appeared plate-like, while their shape became longer and narrower with increasing PLR. The longest crystals could be observed for a PLR of 3.0.

Partial replacement of diammonium hydrogen phosphate in the cement liquid by diammonium citrate had significant influence on the wet compressive strength of the cement cuboids, as Table 2 shows. While for a citrate content of 0.5 M the CS reached a maximum at 64.55 MPa, CS values decreased drastically with further increasing citrate concentration. When diammonium hydrogen phosphate in the cement liquid was completely replaced by diammonium citrate, the resulting samples had a CS of about only 3.77 MPa. The density of the struvite cements decreased continuously with increasing citrate content from 1.88 mg/mm³ for 0 M citrate to 1.48 mg/mm³ for 3.5 M citrate (Table 2). In parallel the paste moldability changed from pasty and moldable to liquid. The setting time according to the Gilmore needle test increased with rising citrate content, however the setting time under normal

Table 1 Wet compressive strength, density and initial setting time according to the Gilmore needle test of struvite cements after 24 h at 37°C and 100% humidity

PLR in g/ml	Compressive strength in MPa	Density in mg/mm ³	Initial setting time in min	Paste moldability
1.0	19.72 ± 3.79**	1.58 ± 0.02	16 ± 1	Very low viscosity, paste-like after 23 min
1.5	41.26 ± 6.78	1.69 ± 0.05	6 ± 1	Low viscosity, paste-like after 7 min
2.0	56.13 ± 10.77*	1.84 ± 0.01	3 ± 1	Initially liquid and pasty after 3.5 min; mouldable after 5 min
2.5	49.36 ± 5.63	1.88 ± 0.05	2 ± 1	Pasty, mouldable after 2 min
3.0	48.10 ± 10.68	1.89 ± 0.02	2 ± 1	High viscosity paste, easy to shape
3.3	45.60 ± 6.95	1.90 ± 0.01	1.5 ± 1	Dry consistency

Cements were prepared from $\text{Mg}_3(\text{PO}_4)_2$ (2 h ground) with a 3.5 M $(\text{NH}_4)_2\text{HPO}_4$ solution as cement liquid. Paste moldability was examined at room temperature

* Significantly ($P < 0.05$) higher than PLR 3.3, 1.5 and 1.0

** Significantly ($P < 0.05$) lower than other values

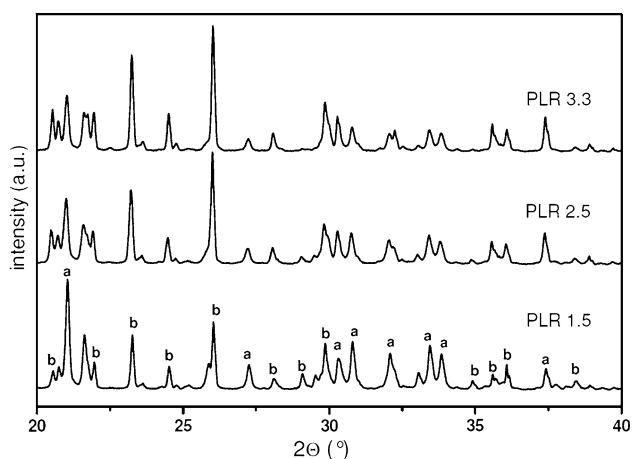


Fig. 1 X-ray diffraction analysis of struvite cements made from $\text{Mg}_3(\text{PO}_4)_2$ and a 3.5 M $(\text{NH}_4)_2\text{HPO}_4$ solution depending on the powder to liquid ratio. The most prominent peaks of *a* struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$, PDF: 003–0240) and *b* farringtonite syn ($\text{Mg}_3(\text{PO}_4)_2$, PDF: 033–0876) are marked

ambient conditions showed a minimum of about 7 min for a citrate content of 0.5 M. Cements with citrate contents of 2 M and above showed a vast increase of the setting time to more than 60 min. Morphological characterization with SEM of the struvite samples showed a noticeable increase in crystallite size with rising citrate content in the cement liquid (Fig. 2). Cements with a citrate content of 0.5 M consisted of one phase with small crystallite sizes and another phase of bigger and thinner crystals (Fig. 2b). In cements with 1.0 and 1.5 M citrate grew even bigger and plate-like and showed a tendency to agglomeration (Fig. 2c, d).

The temperature measurements showed that the application of citrate containing cement liquids increased the maximum setting temperature by up to 18°C (Fig. 3). The initial slopes of the temperature curves were considerably

higher for citrate containing cement liquids than for the one, where only diammonium hydrogen phosphate was used. Furthermore, Fig. 3 shows that higher citrate contents also led to a faster decrease of the temperature towards the physiological value of 37°C. The exact maximum setting temperatures of the different cement liquid compositions were 54.3°C after 469 s for pure $(\text{NH}_4)_2\text{HPO}_4$, 68.3°C after 252 s for 0.5 M citrate, 72.7°C after 170 s for 1.0 M citrate, and 65.6°C after 200 s for 1.5 M citrate.

The injectability tests showed that only cement pastes with a $\text{PLR} \leq 2.0$ could be injected through the syringe. At a PLR of 2.5 it was impossible to inject the paste with maximal manual force even after removing the canula, solid and liquid cement phase separated due to the ‘filter-pressing’ effect [16]. Replacement of diammonium hydrogen phosphate in the cement liquid by diammonium citrate significantly reduced the forces required for cement ejection through the syringe (Fig. 4). The injectability values calculated according to equation 2 are presented in Table 3. There was a huge variation in the behavior of citrate-free cements, which resulted in the big errors. Cements prepared with a PLR of 2.5 could be practically injected completely when they contained 1.5 M citrate, while at PLR 2.0 even 1.0 M citrate was sufficient. The zeta-potentials of farringtonite particles in cement liquids were significantly higher than in pure water (Table 4). However, the zeta-potential values varied only marginally depending on the different compositions of the cement liquids.

4 Discussion

Prior to setting, ceramic biocements can be considered as high viscosity ceramic powder suspensions. Often when these cements that consist only of ceramic particles and

Table 2 Wet compressive strength, density and initial setting time according to the Gilmore needle test of struvite cements after 24 h at 37°C/100 % humidity

$(\text{NH}_4)_2\text{citrate}$ content in M	Compressive strength in MPa	Density in mg/mm^3	Initial setting time in min	Paste moldability
0	$49.36 \pm 5.63^*$	1.88 ± 0.05	2 ± 1	Pasty, mouldable after 2 min
0.5	$64.55 \pm 10.99^*$	1.85 ± 0.01	2 ± 1	Well adaptable with a spatula
1.0	$39.00 \pm 9.73^*$	1.75 ± 0.01	2 ± 1	Initially liquid, pasty and mouldable after 1 min
1.5	$23.96 \pm 7.17^*$	1.72 ± 0.05	7.5 ± 1	Liquid, paste-like after 4 min
2.0	12.90 ± 6.22	1.66 ± 0.10	>60	Liquid, paste-like after 10 min
2.5	11.31 ± 4.05	1.53 ± 0.01	>60	Liquid
3.0	3.87 ± 0.94	1.54 ± 0.10	>90	Liquid
3.5	3.77 ± 1.02	1.48 ± 0.04	>90	Liquid

Cements were prepared from $\text{Mg}_3(\text{PO}_4)_2$ (ground for 2 h) with a mixed solution of $(\text{NH}_4)_2\text{HPO}_4$ and diammonium citrate (total salt concentration 3.5 M) at a PLR of 2.5 g/ml. Paste moldability was examined at room temperature

* Significantly different ($P < 0.05$) from other values

Fig. 2 SEM micrographs of struvite cement morphology after setting with different solutions at PLR 2.5 g/ml for 24 h at 37°C. **a** 3.5 M $(\text{NH}_4)_2\text{HPO}_4$, **b** 3.0 M $(\text{NH}_4)_2\text{HPO}_4$ + 0.5 M $(\text{NH}_4)_2\text{citrate}$, **c** 2.5 M $(\text{NH}_4)_2\text{HPO}_4$ + 1.0 M $(\text{NH}_4)_2\text{citrate}$, **d** 2.0 M $(\text{NH}_4)_2\text{HPO}_4$ + 1.5 M $(\text{NH}_4)_2\text{citrate}$

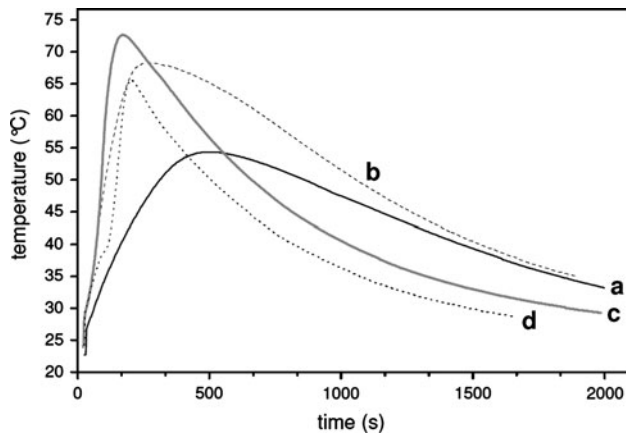
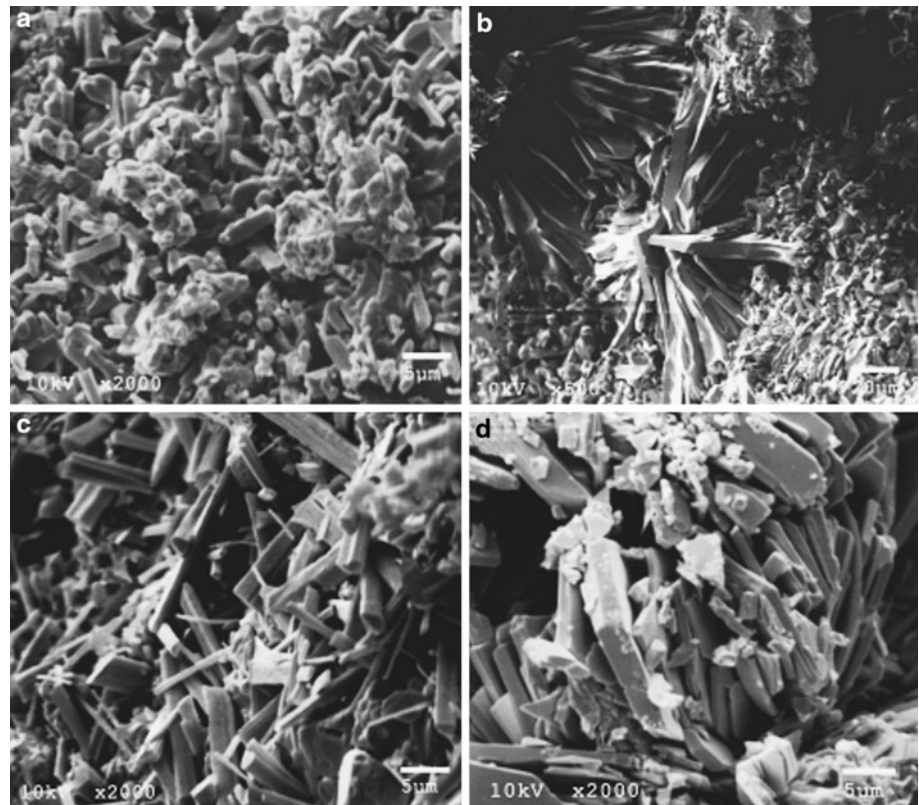


Fig. 3 Temperature evolution during setting of struvite forming biocements formed by the reaction of $\text{Mg}_3(\text{PO}_4)_2$ with various solutions at PLR 2.5 g/ml. **a** 3.5 M $(\text{NH}_4)_2\text{HPO}_4$; **b** 3.0 M $(\text{NH}_4)_2\text{HPO}_4$ + 0.5 M $(\text{NH}_4)_2\text{citrate}$; **c** 2.5 M $(\text{NH}_4)_2\text{HPO}_4$ + 1.0 M $(\text{NH}_4)_2\text{citrate}$; **d** 2.0 M $(\text{NH}_4)_2\text{HPO}_4$ + 1.5 M $(\text{NH}_4)_2\text{citrate}$

water as liquid phase are delivered through a narrow bore needle or canula, a filter-pressing phenomenon can occur, leading to a separation of liquid and solid phase [16]. Although injectable cements can be obtained by decreasing the PLR [17], this often has a detrimental effect on the mechanical properties, since the excess of water leads to an over-all increase of porosity in the cement microstructure [18]. Attempts to improve the injectability properties

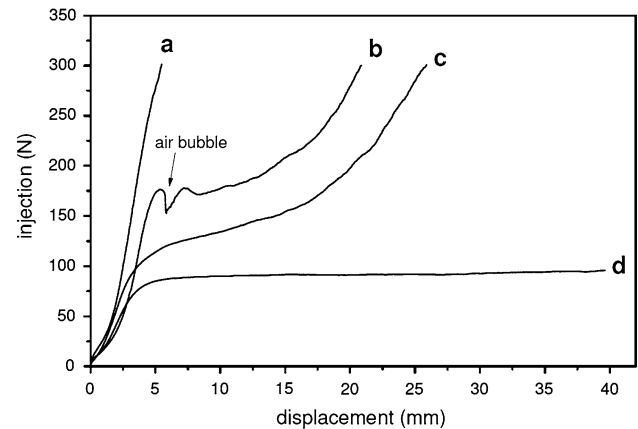


Fig. 4 Force-displacement curves, recorded during ejection of cement pastes through a syringe equipped with a 19 G hypodermic needle. Cements were prepared from $\text{Mg}_3(\text{PO}_4)_2$ using cement liquids at a constant powder to liquid ratio of 2.5 g/ml and the following compositions: **a** 3.5 M $(\text{NH}_4)_2\text{HPO}_4$, **b** 3.0 M $(\text{NH}_4)_2\text{HPO}_4$ + 0.5 M $(\text{NH}_4)_2\text{citrate}$, **c** 2.5 M $(\text{NH}_4)_2\text{HPO}_4$ + 1.0 M $(\text{NH}_4)_2\text{citrate}$, **d** 2.0 M $(\text{NH}_4)_2\text{HPO}_4$ + 1.5 M $(\text{NH}_4)_2\text{citrate}$

include the use of several additives to the liquid phase, such as lactic acid, glycerol, chitosan, citric acid or soluble polymers [19, 20]. Main requirements for additives are non-toxicity as well as the absence of inhibitive effects on the cement setting reaction. These additives work in several ways: they may increase the viscosity of the liquid phase by several orders of magnitude and make the cement

Table 3 : Injectability of cement pastes at powder to liquid ratios of 2.0 and 2.5 g/ml

Cement liquid	Injectability in %	
	PLR 2.0 g/ml	PLR 2.5 g/ml
3.5 M (NH ₄) ₂ HPO ₄	77.67 ± 22.41	19.81 ± 20.65
3.0 M (NH ₄) ₂ HPO ₄ + 0.5 M (NH ₄) ₂ citrate	87.67 ± 2.20	46.90 ± 7.20
2.5 M (NH ₄) ₂ HPO ₄ + 1.0 M (NH ₄) ₂ citrate	98.73 ± 0.10	53.61 ± 0.70
2.0 M (NH ₄) ₂ HPO ₄ + 1.5 M (NH ₄) ₂ citrate	98.92 ± 0.09	98.04 ± 0.82

pastes putty-like or they improve the wettability and increase the surface charge of the cement particles due to an adsorption of ions onto the particle surface. While the latter was previously demonstrated for both apatite and brushite cements, e.g. by using citrates, this principle was not yet applied to struvite forming magnesium phosphate cements, and therefore it was chosen as the main subject of this study. However, firstly the PLR of the cements was varied in order to find the optimal value as a basis for the following experiments with citrate addition.

The influence of PLR variation on the compressive strength of the set cements was not significant for the higher PLR (3.3–2.5 g/ml) values. In general, the CS of struvite cements system is less sensitive to the PLR than calcium phosphate cements, which can be explained by the setting reaction (Eq. 1), which shows that apparently a high amount of water is bound by struvite precipitation during setting. Furthermore an increasing amount of binder liquid increases the amount of diammonium hydrogen phosphate, which promotes the conversion of the reactants to the products. Thus, increasing amounts of water can be compensated to a certain degree, and the CS is maintained over a relatively broad PLR range. Cements set at PLR 2.0 showed the highest compressive strength with 56.13 MPa, which was significantly ($P < 0.05$) higher than PLR 3.3, 1.5 and 1.0 g/ml; CS dropped drastically with further decrease of the PLR and was significantly ($P < 0.05$) lower than all other values for a PLR of 1.0 g/ml. Furthermore, at the lowest PLR of 1.0 the setting time obtained with the Gilmore needle test exceeded the limits of clinical applicability, while very high PLRs resulted in high viscosity and dry consistency of the cements. However, under normal ambient conditions setting times are usually higher, which means that even a PLR of 1.5 is too low for clinical

applications. Cements prepared with PLRs 2.0 and 2.5 represented a good compromise regarding moldability of the pastes, compressive strength and density of the dried cements, with the addition of setting times meeting clinical demands. XRD measurements showed that the transformation of farringtonite to struvite occurred for the whole range of tested PLRs; however, in cements with higher PLR the amount of unreacted farringtonite was higher due to the reduced concentration of phosphate ions in the cement paste.

The addition of citrate ions to the cement liquid had a strong influence on the mechanical properties and the level of injectability of the cement. A low citrate content of 0.5 M significantly ($P < 0.05$) improved compressive strength, while further increase of the citrate concentration resulted in significantly reduced CS of the set cements. This was accompanied by decreasing density and viscosity and an increase of the setting time. At the same time the maximum temperature during the setting reaction was elevated from about 54°C up to 72°C with rising citrate content. Also the maximum temperature peak appeared after shorter times, which indicates an accelerated setting reaction [21].

The flow of phosphate-based cement pastes through a cylinder, in this case the injection needle, can be described by the Hagen–Poiseuille relationship:

$$\Delta Q_i = \frac{\Delta P_i \pi D_n^4}{128 \mu_p L_n} \quad (3)$$

In this equation μ_p is the viscosity of the fluid, D_n is the diameter of the needle, L_n is the length of the needle and ΔP_i is the pressure drop that has to be applied on the paste to obtain a volume flow rate ΔQ_i [22]. Apparently, the needle diameter is a crucial parameter for the judgment of cement paste injectability, which was also shown experimentally by Burguera et al. [23]. As it is therefore essential to perform injectability tests with high gauge size needles, an inner diameter of 800 μm chosen for this study seems appropriate. The injectability of the tested struvite cements was considerably increased by the addition of diammonium citrate to the cement liquid. However, this effect cannot be attributed to the increased negative zeta-potential of the farringtonite particles in cement liquid, because the zeta-potential values were practically the same in all solutions tested, regardless if they consisted of diammonium hydrogen phosphate, diammonium citrate or a mixture of both. A logical explanation may be the fact

Table 4 Zeta-potentials of Mg₃(PO₄)₂ particles in different solutions

	Water	0.05 M (NH ₄) ₂ HPO ₄	0.05 M (NH ₄) ₂ citrate	0.03 M (NH ₄) ₂ HPO ₄ + 0.02 M (NH ₄) ₂ citrate
Zeta-potential (mV)	-19.5 ± 0.2	-40.0 ± 0.6	-37.9 ± 1.4	-41.1 ± 0.6

that the citrate ions are not consumed during the setting reaction. In opposite to the reaction with a cement liquid containing only diammonium hydrogen phosphate, the increased zeta-potential and therefore the increased repulsion between the particles is maintained during the setting of a cement paste containing citrate ions.

A crucial criterion for a biomedical cement application is their cytocompatibility in contact with human cells. Although this was not tested in the current study, struvite forming cements with similar composition demonstrated a high biocompatibility [24, 25]. The activity of osteoblastic cells was higher on struvite in comparison to hydroxyapatite [24], and also osteoclastic cells were shown to grow on struvite cements, followed by active degradation of the cement matrix [25]. Generally, struvite cements show the best degradability, when compared with hydroxyapatite or secondary calcium phosphates like brushite and monetite.

The setting reaction of the struvite cements was found to be an exothermic process with a temperature rise of the cements to 58–72°C during setting. Similar to polymeric cements based on polymethylmethacrylate (PMMA, T_{\max} during setting up to 80–100°C) [26] this may cause protein denaturation and thermal tissue necrosis. However, the amount of ceramic cement used in most clinical situations is much smaller than in our study, in most cases defects are filled with less than 5 cm³ cement paste. Also, in contrast to the testing regime in this study, which included an effective insulation of the cement paste during setting, an improved heat flow under in vivo conditions between the cement and the surrounding tissue will likely decrease the maximum temperatures and hence avoid thermal tissue damage. However, as discussed by Mestres et al. [21], there might be a need for exothermically setting cements, e.g. for the treatment of vertebral bone tumours, where besides the mechanical stabilisation of the defect, the local heat release of an exothermically setting cement may have a hyperthermal effect on the tumour.

5 Conclusions

The mechanical properties and the level of injectability of magnesium phosphate cements were examined, regarding the influence of variations in PLR and diammonium citrate content. The aim was to adjust these parameters in order to obtain an optimal combination of fast setting, moderate setting temperature, early strength development, and good injectability. The variation of PLR showed good compressive strength for values above 1.5 g/ml. The paste moldability and the setting time appeared to be most adequate for clinical applications, when the PLR was adjusted to 2.0–2.5 g/ml; however these cements were practically not injectable. Therefore, the experiments with partial

replacement of diammonium hydrogen phosphate by diammonium citrate were restricted to a PLR of 2.5. High citrate contents led to decreasing compressive strengths, hence only cement powders containing 0.5 or 1.0 M citrate in the cement liquid should be taken into account for clinical applications, also regarding their appropriate setting time of approximately 7 min. The injectability of the cement paste was vastly improved for these liquid compositions, hence the addition of citrate can be considered as the right way to achieve clinical applicability of the cements. The setting temperature was slightly increased as compared to citrate-free cements. So far it can be concluded that a PLR of 2.5 and citrate content in the binder liquid of 0.5 mol/l provide the best combination of good injectability, high compressive strength, appropriate setting time and acceptable setting temperature increase.

References

1. Bohner M. Physical and chemical aspects of calcium phosphates used in spinal surgery. *Eur Spine J.* 2001;10:114–21.
2. Pittet C, Lemaître J. Mechanical characterization of brushite cements: a Mohr circles' approach. *J Biomed Mater Res.* 2000; 53(6):169–780.
3. Theiss F, Apelt D, Brand BA, Kutter A, Zlinszky K, Bohner M, Matter S, Frei C, von Auer JA. Biocompatibility and resorption of a brushite calcium phosphate cement. *Biomaterials.* 2005;26(21): 4383–94.
4. Oberle A, Theiss F, Bohner M, Müller J, Kastner SB, Frei C, Zlinszky K, Wunderlin S, von Auer JA, von Rechenberg B. Investigation about the clinical use of brushite-and hydroxylapatite-cement in sheep. *Schweiz Arch Tierh.* 2005;147(11): 482–90.
5. Kuemmerle JM, Oberle A, Oechslin C, Bohner M, Frei C, Boeckel I, von Rechenberg B. Assessment of the suitability of a new brushite calcium phosphate cement for cranioplasty—an experimental study in sheep. *J Cranio-Maxillofac Surg.* 2005; 33(1):37–44.
6. Bohner M. pH variations of a solution after injecting brushite cements. *Key Eng Mater.* 2000;192–195:813–6.
7. Coe FL, Evan A, Worcester E. Kidney stone disease. *J Clin Invest.* 2005;115(10):2598–608.
8. Rahaman MS, Mavinic DS, Bhuiyan MIH, Koch FA. Exploring the determination of struvite solubility product from analytical results. *Environ Tech.* 2006;27(9):951–61.
9. Bhuiyan MI, Mavinic DS, Beckie RD. A solubility and thermodynamic study of struvite. *Environ Technol.* 2007;28(9):1015–26.
10. Driessens FCM, Boltong MG, Wenz R, Meyer J. Calcium phosphates as fillers in struvite cements. *Key Eng Mater.* 2005; 284–286:161–4.
11. Hall DA, Stevens R, El Jazairi B. Effect of water content on the structure and mechanical properties of magnesia-phosphate cement mortar. *J Am Ceram Soc.* 1998;81(6):1550–6.
12. Sarkar AK. Hydration dehydration characteristics of struvite and dittmarite pertaining to magnesium ammonium phosphate cement systems. *J Mater Sci.* 1991;26(9):2514–8.
13. Vormdran E, Ewald A, Müller FA, Zorn K, Kufner A, Gbureck U. Formation and properties of magnesium–ammonium–phosphate hexahydrate biocements in the Ca–Mg–PO₄ system. *J Mater Sci Mater Med.* 2011;22:429–36.

14. Banfill PFG. The rheology of fresh cement and concrete—a review. In: Grieve G, Owens G, editors. Proceedings on the 11th international cement chemistry congress, Durban, 2003, pp 13.
15. Gbureck U, Barralet JE, Grover LM, Spatz K, Thull R. Ionic modification of calcium phosphate cement viscosity. Part I: hypodermic injection and strength improvement of apatite cements. *Biomaterials*. 2004;25:2187–95.
16. Baroud G, Bohner M, Heini P, Steffen T. Injection biomechanics of bone cements used in vertebroplasty. *Biomed Mater Eng*. 2004;14:487–504.
17. Khairoun I, Boltong MG, Driessens FCM, Planell JA. Some factors controlling the injectability of calcium phosphate bone cements. *J Mater Sci Mater Med*. 1998;9:425–8.
18. Ishikawa K, Asoaka K. Estimation of ideal mechanical strength and critical porosity of calcium phosphate cement. *J Biomed Mater Res*. 1995;29:1537–43.
19. Leroux L, Hatim Z, Freche M, Lacout JL. Effects of various adjuvants (lactic acid, glycerol and chitosan) on the injectability of a calcium phosphate cement. *Bone*. 1999;25(2):31–4.
20. Ginebra MP, Rilliard A, Fernandez E, Elvira C, San Roman J, Planell JA. Mechanical and rheological improvement of a calcium phosphate cement by the addition of a polymeric drug. *J Biomed Mater Res*. 2001;57(1):113–8.
21. Mestres G, Ginebra MP. Novel magnesium phosphate cements with high early strength and antibacterial properties. *Acta Biomater*. 2011;7(4):1853–61.
22. Bohner M, Baroud G. Injectability of calcium phosphate pastes. *Biomaterials*. 2005;26(13):1553–63.
23. Burguera EF, Xu HHK, Sun L. Injectable calcium phosphate cement: effects of powder-to-liquid ratio and needle size. *J Biomed Mater Res*. 2008;84B:493–502.
24. Ewald A, Helmschrott K, Knebl G, Mehrban N, Grover LM, Gbureck U. Effect of cold-setting calcium- and magnesium phosphate matrices on protein expression in osteoblastic cells. *J Biomed Mater Res*. 2010;96B:326–32.
25. Großardt C, Ewald A, Grover LM, Barralet JE, Gbureck U. Passive and active in vitro resorption of calcium and magnesium phosphate cements by osteoclastic cells. *Tissue Eng*. 2010;16A(12):3687–95.
26. Li C, Mason J, Yakimicki D. Thermal characterization of PMMA-based bone cement curing. *J Mater Sci Mater Med*. 2004;15(1):85–9.