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Early-stage macroporosity enhancement in calcium phosphate cements by inclusion of poly(N-vinylpyrrolidone) particles as a porogen

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ABSTRACT

The incorporation of poly(DL-lactic-co-glycolic acid) (PLGA) particles into calcium phosphate cements (CPCs) is an effective strategy to enhance CPC macroporosity and degradation. However, bone regeneration is hindered until hydrolytic PLGA degradation starts a few weeks after implantation. Additionally, CPC and CPC/PLGA injectability and cohesion are suboptimal. In the current study, poly(N-vinylpyrrolidone) (PVP), a water-soluble polymer, was incorporated as a porogen in CPC and CPC/PLGA composites to enhance handling properties and early-stage macroporosity formation. Further, the effect of PVP molecular weight (Mw) and particle size was studied. The results showed that PVP incorporation increased both injectability and cohesion of the CPC pastes, especially with addition of high Mw PVP. Moreover, the *in vitro* degradation studies revealed that incorporation of PVP induced an initial mass loss during the first week of incubation. In combination with PLGA, small PVP particles induced a higher mass loss at an early stage than large PVP particles, but this effect was no longer apparent after 4 weeks of incubation. In contrast, the incorporation of low Mw PVP had a stronger effect on *in vitro* degradation in the long term compared to high Mw. Finally, the presence of PLGA porogens appeared to be necessary for adequate CPC degradation.

1. Introduction

The regeneration of bone defects has been extensively studied in maxillofacial and orthopedic surgery. Bone defects can arise from endogenous (e.g. cysts, tumors, or congenital deformities) or exogenous causes (e.g. injuries from accidents) and its regeneration may require the use of bone regeneration techniques [1]. The gold standard in bone regeneration techniques is the use of bone grafts and, specifically, the use of autografts. However, autografts have different drawbacks, such as low availability or secondary surgery site and donor site morbidity, among others [2]. Synthetic bone grafts, such as polymers or ceramics, are a promising alternative as they are off-the-shelf available. On the other hand, one of the current demands in reconstructive surgery is the degradability of bone substitutes [3,4]. Bone substitutes are destined to dissolve and degrade postoperatively synchronized with bone healing periods, so that they do not hinder new bone formation. Therefore, the design and construction of degradable bone substitutes is of high importance.

Calcium phosphate (CaP)-based materials are one of the most used synthetic bone substitutes. Of those, calcium phosphate cements (CPCs) seem the most appealing, due to their injectable and moldable nature. CPCs are composed of a powder and a liquid phase that, when mixed, form a paste that sets and hardens into a solid mass. Depending on the end product of the setting reaction, CPCs can be classified in apatitic and brushite cements. While brushite cements have a higher degradation rate than apatitic CPCs, the latter have a more similar mineral phase to bone and superior mechanical properties [5–7]. The downside of apatitic CPCs is that they are slowly biodegraded and thus hamper the replacement of the CPC with native bone tissue [8–11]. Therefore, the degradation of apatitic CPCs has to be enhanced so that their replacement with native bone tissue can occur.

In order to accelerate apatitic CPC degradation, macroporosity can

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be introduced. It is known that the presence of macroporosity can enhance the active and passive degradation of CPCs by allowing cell migration and proliferation into the CPC matrix as well as by increasing the surface area [12–14]. Consequently, numerous strategies have been pursued to increase the macroporosity of apatitic CPCs. The use of polymeric microparticles, also known as polymeric porogens, into apatitic CPC is among the most extensively used approaches. Poly(D,L-lactic-co-glycolic acid) (PLGA) has shown to be a great polymeric candidate; its hydrolytic degradation not only creates a macroporous structure within the CPC, but also produces acidic monomers (i.e. lactic and glycolic acid) that further enhance the degradation of the CPC matrix [15,16]. However, bone ingrowth into the CPC is delayed until PLGA degradation starts ~2 weeks after implantation [17].

Water-soluble porogens, on the other hand, create a nearly immediate macroporous structure. Nevertheless, the addition of water-soluble polymers to CPCs would only enhance degradability by creating macroporosity and increasing the surface available to degrade, but it would not affect the structure of the CPC matrix itself. Previous work focused on the combination of a water-soluble porogen, such as sucrose and PLGA, to obtain a multimodal porogen system with fast macroporosity created by the sucrose and late stage macroporosity as well as CPC degradation enhancement due to PLGA degradation [18,19]. However, sucrose dissolution occurred too early, and, therefore, it might fully dissolve before CPC setting is completed which can cause matrix densification [20]. Furthermore, the incorporation of sucrose in CPC/PLGA systems increased the final setting time to ~80 min and reduced the CPC paste cohesion.

A solution for this problem can be the use of water-soluble polymers, as their dissolution can be tuned. Poly(N-vinylpyrrolidone) (PVP) is a water-soluble polymer that is already being used on a wide-scale for commercial purposes in the food, cosmetic or pharmaceutical industries. Also, PVP has been used as a lubricating agent for injections, making it an excellent material to add to CPC regarding the injectability properties [21–23]. Moreover, PVP has been previously incorporated into the liquid phase of CPCs to act as a cohesion enhancer [24].

Therefore, we hypothesized that the use of a dual porogen system combining the water-soluble polymer PVP with hydrolytically degrading PLGA would 1) improve CPC paste injectability and cohesion, and 2) create an early macroporous structure due to PVP dissolution and a later stage CPC degradation due to PLGA hydrolytic degradation. Additionally, we hypothesized that porogen size and molecular weight (Mw) would have an effect on CPC handling properties and would affect PVP dissolution. For this, handling properties were explored and *in vitro* degradation experiments of CPCs including PVP particles of different sizes and Mw with and without PLGA were performed.

2. Materials and methods

2.1. Materials

CPC powder consisted of 100 % alpha tricalcium phosphate (α-TCP) (CAM Bioceramics BV; Leiden, The Netherlands). The liquid phase for the cement preparation was sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2\ H_2O$) and was purchased from Merck (Darmstadt, Germany). PLGA (lactic:glycolic acid ratio 50:50; molecular weight of 17 kDa; acid terminated) was used (Corbion Purac, Gorinchem, The Netherlands) in the form of microparticles (mean particle size of approximately 60 μm). Poly(N-vinylpyrrolidone) (PVP) with a Mw of 10 kDa was purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands) and sieved to a particle size of > 40 μm (S-lMw, mean particle size of ~50 μm). PVP with a Mw of 360 kDa was purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands) and sieved to a particle size of < 100 μm (S-hMw, mean particle size of ~50 μm) and > 100 μm (L-hMw, mean particle size of ~200 μm). The size distribution of all three types of PVP was determined by image analysis. The different PVP particles were observed with an optical microscope (Leica Microsystems AG, Wetzlar, Germany). Subsequently, size distribution was determined from 250 PVP particles of each group by digital image software (Fiji 1.51n) [25].

Table 1
Material components of the various CPC formulations.

Abbreviation	α-TCP[a] (wt.%)	PLGA (wt.%)	PVP (wt.%) (type)	LPR
CPC	100	0	0	0.5
CPC_{Lh}	80	0	20 (L-hMw)	0.3
CPC_{Sh}	80	0	20 (S-hMw)	0.3
CPC_{Sl}	80	0	20 (S-lMw)	0.3
CPC/PLGA	60	40	0	0.415
$CPC/PLGA_{Lh}$	48	32	20 (L-hMw)	0.23
$CPC/PLGA_{Sh}$	48	32	20 (S-hMw)	0.23
$CPC/PLGA_{Sl}$	48	32	20 (S-lMw)	0.23

[a] α-TCP, alpha tricalcium phosphate; PLGA, poly(DL-lactic-co-glycolic acid); PVP, poly(N-vinylpyrrolidone); LPR, liquid-to-powder ratio.

2.2. Preparation of CPC formulations

PVP with different particle size and Mw and/or PLGA particles were added to α-TCP in different ratios (Table 1). The different CPC formulations were mixed with 4 wt/vol% $NaH_2PO_4{\cdot}2\ H_2O$. The liquid to powder ratio (LPR) of each CPC/PLGA/PVP formulation was determined and optimized according to the consistency of the paste. After LPR optimization for the different CPC formulations, pre-set composites were fabricated for use in the degradation study. In all PVP containing formulations, 20 wt.% of PVP particles were added. CPC:PLGA ratio was kept constant to 60:40. After addition of the liquid phase, the powder mix was vigorously stirred using a spatula. Hereafter, the cement paste was molded into a Teflon mold (Ø 9 mm × 4.5 mm) using a spatula. Afterwards, the composites were left overnight at room temperature. Subsequently, samples were removed from the mold and freeze-dried.

2.3. Physicochemical characterization of CPCs

2.3.1. Setting time

Initial and final setting times were determined using Gillmore needles (ASTM C266). With that purpose, the different CPC formulations were mixed with the setting liquid at the appropriate LPR and molded into holes (6 mm diameter and 12 mm of height) of a bronze block placed in a water bath at 37 °C. Periodically, the light Gillmore needle (i.e. for initial setting time) or the heavy Gillmore needle (i.e. for final setting time) were placed onto the surface of the molded CPC. Setting times were recorded when the needles were not able to mark the CPC with a complete circular indentation. (n = 3).

2.3.2. Injectability analysis

For injectability analysis, 1 g of each CPC formulation was mixed with setting liquid at the appropriate LPR. The resulting paste was introduced in a 2.5 mL syringe with a nozzle orifice of 2 mm in diameter (Terumo Europe N.V., Leuven, Belgium) and directly extruded. The extrusion time was kept at 1:30 min to avoid interference of extrusion due to setting time. The injectability (%) was then calculated using Eq. 1., as suggested by Qi et al. (2009) [26].

$$\%\,\text{Injectability} = 100 - \frac{m_n}{m_i} \times 100\% \quad (1)$$

where m_n is the mass of CPC remaining in syringe after injection (g) and m_i is the mass of CPC before injection (g). (n = 3).

2.3.3. Cohesion of the cements

In order to quantify the cohesion, CPC pastes were injected into a solution of phosphate-buffered saline (PBS; pH 7.4; Gibco®, Thermo Fischer Scientific, Waltham, MA) heated to 37 °C. Directly upon

injection, CPC pastes were photographed and graded on its degree of particulate cloud formation (PC) and fragmentation (F) as specified in Table A1 and A2 (Appendix A). Cohesion score was determined following Eq. 2 as described before [27].

$$\text{Cohesion score} = \frac{PC + F}{2} \quad (2)$$

To further quantify the quality of cohesion a washout test was performed as described before [18]. Briefly, the different CPC formulations were mixed with the liquid phase and placed in tissue specimen bags (pore size 170 mm; Thermo Scientific), which were immediately placed in Falcon tubes containing 15 mL PBS. Subsequently, the Falcon tubes were placed on a shaking table for 4 h at 120 rpm in an incubator at 37 °C. After 4 h, the tissue specimen bags were carefully removed from the PBS and both the tissue bags and the Falcon tube containing the PBS were freeze-dried. The weight of both the CPC remaining in the bag (W_{CPC}) and the washed-out particles in the tube (W_{washed}) were determined and the wash out % was calculated using Eq. 3. (n = 3)

$$\%\,\text{washed} = \frac{W_{washed}}{W_{washed} + W_{CPC}} \times 100\% \quad (3)$$

2.3.4. X-ray diffraction analysis

CPCs of each formulation were incubated for 7 days in PBS at 37 °C to allow full phase transformation. After 7 days, samples were freeze-dried overnight, ground to powder and analyzed by powder X-ray diffraction (XRD; X'Pert^3 Powder, PANalytical, Almelo, The Netherlands) to determine the crystal phase of the cement composites. XRD spectra were registered with 2θ from 10° to 60°, a step size of 0.02° and a counting time of 1 s. XRD spectra of α-TCP and hydroxyapatite (HA) powders were used as control.

2.4. In vitro degradation studies

To study the degradation of the composites, set samples (n = 4 per group) were placed in 10 ml PBS and incubated at 37 °C for 1, 2, 4 and 6 weeks. Moreover, a short-term degradation study was performed (n = 4 per group), where CPC/PVP and CPC/PLGA/PVP formulations were incubated in PBS at 37 °C for 1, 2, 4 and 7 days. After each time point, samples were removed from the PBS and freeze-dried overnight. The remaining material of the samples was calculated using Eq. 4 below:

$$\%\,\text{Remaining material} = 100 - \frac{m_i - m_n}{m_i} \times 100\% \quad (4)$$

where m_n is the mass of the sample at t = n (g) and m_i is the mass of the sample at t = 0 (g).

Calcium release and pH were calculated after 1, 2, 4 and 6 weeks of incubation. The calcium release was evaluated by the o-Cresolphtalein-Complexone (OCPC) assay, analyzing the samples at a wavelength of 570 nm (BioTek®, Gen5™). This is assay was used as a quantification of CPC degradation. pH measurements were performed with a pH electrode (Orion, Sigma Aldrich) to quantify PLGA degradation.

2.5. Porosity evaluation

Total and macro-porosity were evaluated by the burn-out method as described before [28]. The purpose of this method was to burn the polymeric porogens out of the CPC so a porous structure would be left. Briefly, set CPCs were subjected to a heating protocol in order to eliminate the organic components of the cements. The samples were first heated at a rate of 1.67 °C min^{-1} until reaching 650 °C. Afterwards, samples were maintained at this temperature for 2 h, and finally temperature was decreased at a rate of 1.67 °C min^{-1} until reaching room temperature. After burning out the polymeric particles, samples were weighed, and Eqs. (5) and (6) were used for the derivation of the total and macro-porosity. (n = 3)

$$\%\,\text{Total porosity}\ (\varepsilon_{tot}) = \left(1 - \frac{m_{burnt}}{V \times \rho_{HA}}\right) \times 100 \quad (5)$$

$$\%\,\text{Macroporosity}\ (\varepsilon_{macro}) = \left(1 - \frac{m_{burnt}}{m_{micro}}\right) \times 100 \quad (6)$$

where m_{burnt} is the average mass macroporous sample (after burning out the polymers, g), m_{micro} is the average mass of the microporous CPC sample (g), V is the volume of the sample (cm^3) and ρ_{HA} is the density of the samples (g/cm^3). Macroporosity was also observed by scanning electron microscopy (SEM, Zeiss Sigma 300) after 1 week of incubation in PBS. Prior to SEM examination, samples were sputter coated with gold. Images were taken at an accelerating voltage of 3 kV under high vacuum.

2.6. Statistical analysis

Graphpad Prism 5 (GraphPad Software Inc., San Diego, CA) was used to analyze the data. Continuous data are expressed as means ± standard deviations (SD). Statistical differences were only assessed comparing each sample to their respective control (CPC or CPC/PLGA) and between samples with different PVP particle size or Mw. Significant differences between compared groups were determined using analysis of variance with Tukey's multiple comparison tests. Results were considered significant at p values lower than 0.05 ($p < 0.05$).

3. Results

3.1. Characterization of PVP particles

Figure B1a, b and c (Appendix B) depict a representative image of PVP L-hMw, S-hMw and S-lMw, respectively. Fig. 1d shows the frequency distribution of the different groups. L-hMw showed a significantly larger ($p < 0.001$) particle size (188 ± 87 μm) than S-hMw and S-lMw. On the other hand, both groups with small particles (S-hMw and S-lMw) showed comparable particle sizes ($p > 0.05$; 50 ± 24 and 46 ± 21 μm).

3.2. Physicochemical characterization of CPCs

3.2.1. Handling properties

Initial and final setting times of the CPC/PVP and CPC/PLGA/PVP formulations can be observed in Fig. 1a and b. The initial setting time of control groups (CPC, 1.8 ± 0.1 min and CPC/PLGA, 3.0 ± 0.3 min) was significantly lower compared to PVP containing groups. PVP particle size nor Mw had a significant impact on initial setting time. Similarly, the final setting time of control groups (CPC, 4.9 ± 0.1 min and CPC/PLGA, 13.7 ± 1.6 min) was significantly lower compared to PVP containing groups. PVP particle size did not have a significant impact on final setting time, but CPC/PLGA$_{Sh}$ had a significantly lower final setting time than CPC/PLGA$_{Sl}$ ($p < 0.001$).

Fig. 1c reveals that paste injectability significantly increased with the addition of all PVP types compared to CPC to ~85 % ($p < 0.01$). In PLGA-containing groups, on the other hand, this effect was not clearly observed and only the addition of low Mw PVP significantly improved injectability ($p < 0.01$).

Figs. 1d and e represent the cohesion of the different CPC pastes. Addition of PVP significantly improved cohesion compared to CPC ($p < 0.001$ when adding high Mw PVP and $p < 0.01$ when adding low Mw PVP) and to CPC/PLGA ($p < 0.001$). PVP particle size did not have a significant impact on cohesion, but high Mw PVP showed significantly lower cohesion values than low Mw PVP ($p < 0.001$ and $p < 0.05$ for PLGA-free and PLGA-containing groups, respectively).

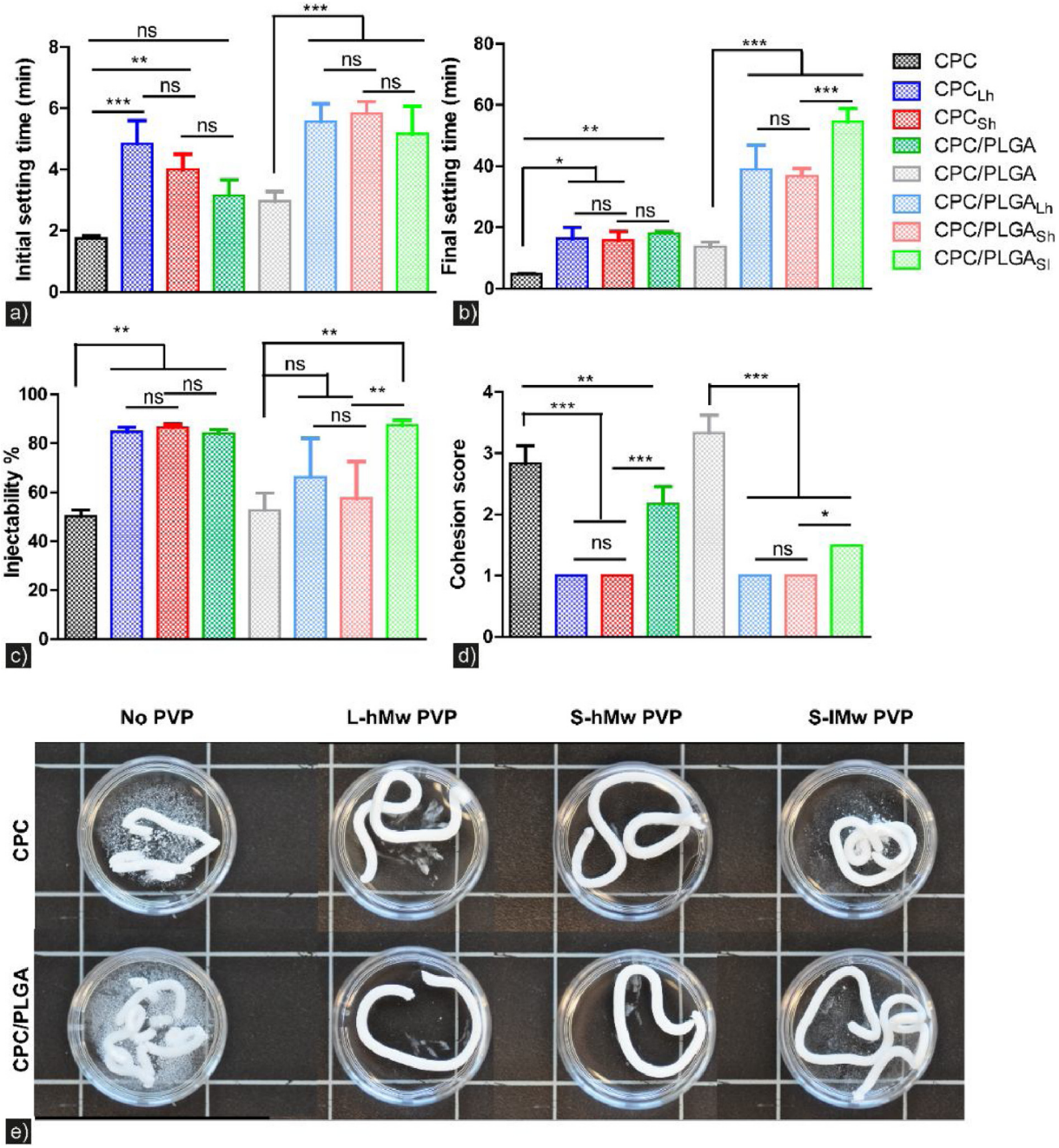

Fig. 1. Handling properties of CPC composites: (a) initial and (b) final (min) setting times; (c) injectability % and (d) cohesion score of the different cement formulations (n = 3); *p < 0.05, **p < 0.01, and ***p < 0.001; ns: not significant differences; error bars represent standard deviation (SD). (e) Representative digital images of CPC composites injected into PBS heated to 37 °C used to calculate the cohesion score.

3.2.2. X-ray diffraction analysis

Fig. 2 shows the powder XRD-patterns of α-TCP and HA powder and all CPC-porogen formulations. After the samples were incubated in PBS for 7 days, the α-TCP fully transformed into HA. In all study groups, the characteristic peaks of α-TCP [29] disappeared and the major reflection peaks of HA were revealed (at 2Θ = 25.9°, 31.7°, 32.2°, 39.8°,46.7°, 49.6° and 53.3°) [30]. Addition of PLGA and PVP did not affect phase transformation.

3.3. In vitro degradation studies

In vitro degradation of set CPC samples was assessed using mass loss measurements (Fig. 3). The remaining material (%) of the PLGA-free composites, as a function of soaking time, is given in Fig. 3a and b. With no PLGA, pure ceramic (CPC) barely degraded and all the ceramic was still present after 6 weeks of incubation. Inclusion of PVP particles resulted in significantly less material remaining than CPC at all time points ($p < 0.001$). Mass loss started after 1 day of incubation and gradually continued up to week 4 and the remaining material (%) for CPC$_{Lh}$, CPC$_{Sh}$ and CPC$_{Sl}$ was 89.7 ± 1.0, 89.6 ± 0.1 and 87.2 ± 0.7 %, respectively. PVP particle size had no significant effect on degradation ($p > 0.05$). Low Mw PVP, on the other hand, significantly increased mass loss after 4 and 7 days of incubation ($p < 0.001$).

The remaining material (%) of the PLGA-containing composites, as a function of soaking time, is shown in Fig. 3c and d. CPCs containing PLGA revealed considerable mass loss during the 6 weeks of incubation and the minimum amount of remaining material was 34.6 ± 2.3 % for CPC/PLGA$_{Sl}$. CPC/PLGA-only samples started to degrade after 1 week

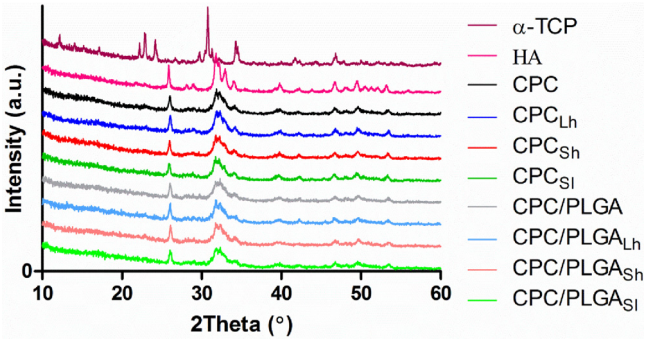

Fig. 2. XRD patterns of α-TCP and HA powders (controls) and the different CPC-porogen formulations after 7 days of incubation, showing transition of α-TCP to HA upon immersion in aqueous solution for all formulations.

of incubation. Inclusion of PVP particles resulted in significantly less remaining material compared with CPC/PLGA specimens, especially at early time points. Incorporation of small PVP particles resulted in significantly less remaining material after 2 weeks of incubation compared to incorporation of large PVP particles ($p < 0.001$). Incorporation of hMw PVP, on the other hand, had no significant effect after 2 weeks of incubation ($p > 0.05$), but incorporation of lMw PVP enhanced mass loss after 4 and 6 weeks of incubation.

PLGA degradation results in formation of acidic by-products (i.e. lactic and glycolic acid), which were quantified by means of pH change (Fig. 3e). CPC and CPC/PVP composites revealed a marginal pH decrease. In contrast, CPC/PLGA and CPC/PLGA/PVP composites decreased the pH of the incubation medium for all experimental groups. CPC/PLGA showed a substantial pH decrease after 2 weeks compared to PVP-containing groups ($p < 0.001$). PVP particle size and Mw had a significant impact on pH decrease at an early stage (i.e. weeks 1 and 2 of incubation; $p < 0.001$).

Also, CPC degradation was quantified by means of calcium (Ca^{2+}) release (Fig. 3f). For CPC and CPC/PVP composites, Ca^{2+} release was limited. After 1 week of incubation, all samples released ~70 µg of Ca^{2+}. Between week 1 and week 6 no further significant release was observed for CPC, CPC$_{Lh}$ and CPC$_{Sh}$ ($p > 0.05$), only CPC$_{Sl}$ showed a slight increase in release up to week 4 ($p < 0.001$). Ca^{2+} release from

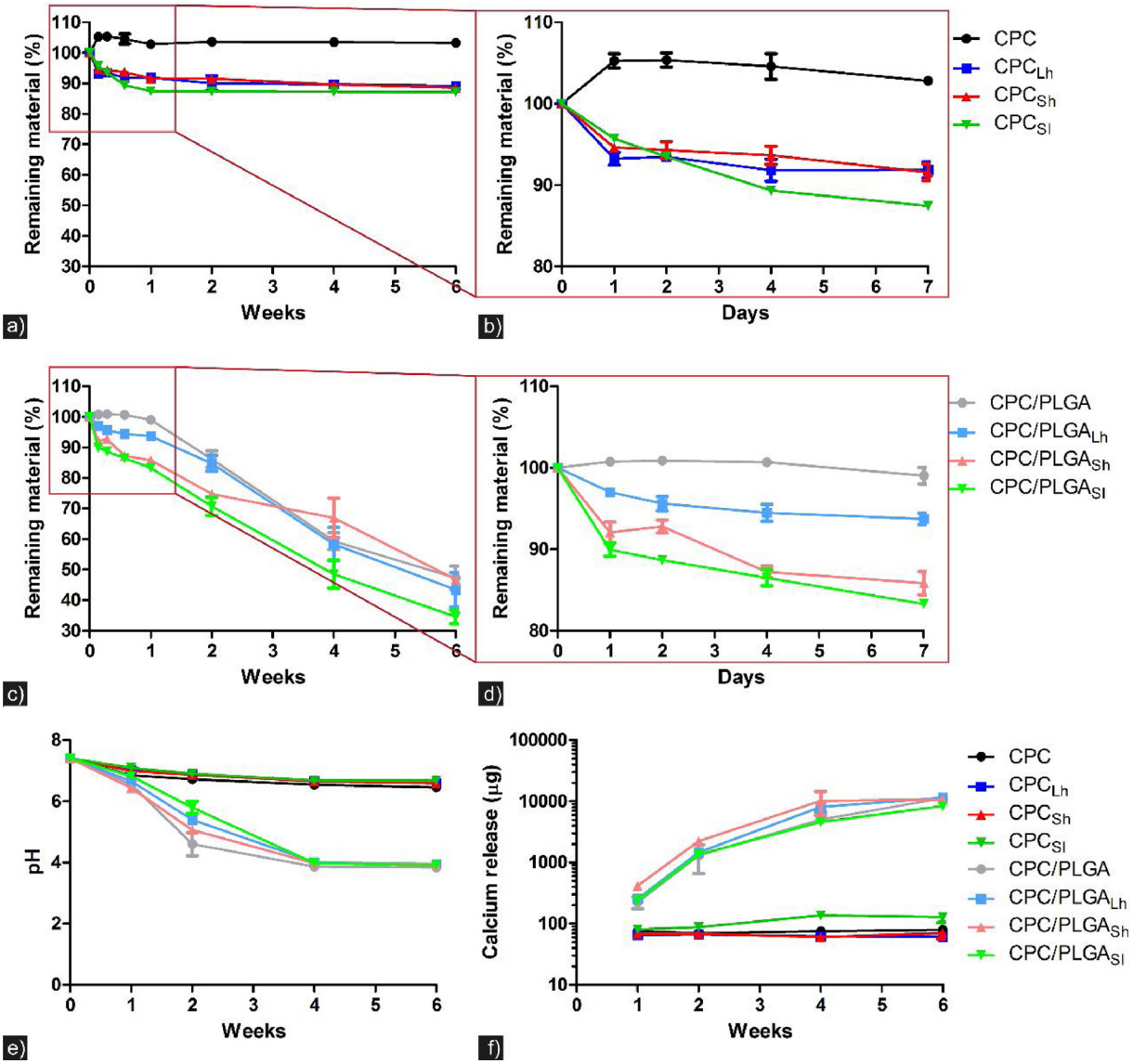

Fig. 3. Degradation of CPC composites. (a) Mass loss of PLGA-free groups represented by the remaining material as a function of degradation time and (b) magnification of the mass loss in week 1. (c) Mass loss of PLGA-containing groups represented by the remaining material as a function of degradation time and (d) magnification of the mass loss in week 1. (e) pH and (f) calcium release of each cement formulation to the surrounding PBS. Error bars represent SD.

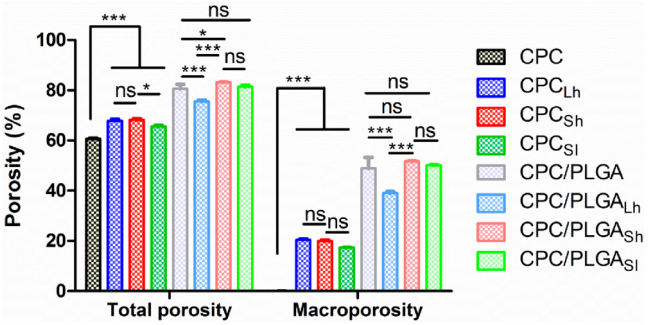

Fig. 4. Total porosity and macroporosity (%) of CPC composited measured by the burn-out method. Error bars represent SD.

CPC/PLGA and CPC/PLGA/PVP samples was significantly higher than CPC and CPC/PVP at all time points ($p < 0.001$). Similar to pH measurements, PVP particle size and Mw had a stronger effect at early time points (i.e. weeks 1 and 2, $p < 0.001$) than at later time points.

3.4. Porosity evaluation

SEM images (Figure B1) were taken after 1 week of incubation. It was observed that PVP had already dissolved while PLGA was still present. Total porosity and macroporosity of the different composites were studied and are shown in Fig. 4. Total porosity of CPC was 60.6 ± 0.3 %. Inclusion of PVP particles significantly increased the total porosity ($p < 0.001$) to 67.8 ± 0.6, 68.2 ± 0.5 and 65.6 ± 0.5 % for CPC$_{Lh}$, CPC$_{Sh}$ and CPC$_{Sl}$, respectively. High-Mw PVP significantly increased total porosity ($p < 0.05$). On the other hand, macroporosity was also significantly increased ($p < 0.001$) for CPC/PVP composites in comparison to CPC, but PVP particle size or Mw did not affect macroporosity ($p > 0.05$). CPC/PLGA composites showed a higher total porosity than CPC composites. CPC/PLGA, CPC/PLGA$_{Lh}$, CPC/PLGA$_{Sh}$ and CPC/PLGA$_{Sl}$ had a total porosity of 80.7 ± 1.8, 75.5 ± 0.6, 83.2 ± 0.2 and 81.4 ± 0.7 %, respectively. Similarly, macroporosity values followed a similar trend to total porosity values.

4. Discussion

The aim of our study was to investigate the potential use of the water-soluble porogen PVP to rapidly achieve macroporosity formation within CPC and CPC/PLGA composites. Additionally, the effect of PVP porogen particle size and molecular weight (Mw) was evaluated. Two different particles sizes (large and small, ~50 and ~200 μm, respectively) and two different Mw (high and low, 360 and 10 kDa, respectively) were selected and their effect of on the physicochemical, handling, and *in vitro* degradation characteristics of CPC and CPC/PLGA composites was assessed. Our results revealed that the initial and final setting time of CPC and CPC/PLGA composites were increased upon the incorporation of PVP, but the phase transformation to hydroxyapatite was not hindered. PVP incorporation improved CPC injectability and cohesion. Furthermore, the *in vitro* degradation studies showed that the incorporation of PVP porogens increased mass loss at an early stage compared to CPC and CPC/PLGA.

The physicochemical properties and handling properties (i.e. setting times, injectability and cohesion) of the different composites were determined. Initial setting time has been described as the clinical window during which it is possible to inject the CPC and mold it to fill the dimensions of the defect, while final setting time indicates the time needed before the wound can be closed. Ideally, initial setting time should be between 3 and 8 min and final setting time should not exceed 15 min [31]. Here, initial setting times complied with these specifications, but final setting times exceeded this requirement, especially when PLGA and PVP porogens were combined within one CPC formulation. It has been described that an increase in the salt concentration of the liquid phase could lead to shorter setting times [31,32]. In order to decrease final setting time a preliminary study was performed with S-lMw PVP by modifying the liquid phase concentration from 4 wt/vol % to 20 wt/vol % aqueous solution of $NaH_2PO_4{\cdot}2\ H_2O$. As no clear benefits were observed (data not shown), 4 wt/vol % was chosen for the rest of the study for standardization purposes. Others have described that the addition of water-soluble porogens (i.e. sucrose and glucose) significantly increases final setting time, which limits their clinical applicability [18,33,34]. On the other hand, while those studies showed final setting times close to 50 min (i.e. for glucose) [33] and of ~35 min and ~80 min (i.e. for sucrose without and with PLGA, respectively) [18], the currently observed increase upon addition of PVP porogens was evidently lower. The increase in setting time is likely related to the higher hydrophilicity of the water-soluble porogens compared to the CaP ceramic, which extracts water from the cement and results in increased setting time [34,35]. In addition, the use of low Mw PVP caused higher final setting times than high Mw PVP. This is possibly due to the faster dissolution rate of low Mw PVP, which consumes more water [36]. This hypothesis is supported by the work of Majekodunmi and Deb (2007), who incorporated another water-soluble polymer and reported a similar influence of molecular weight [37]. This increase in final setting time upon the addition of PLGA and PVP porogens did not hamper the phase transformation from α-TCP to HA.

Cement injectability generally was improved by the addition of PVP. All CPC/PVP composites as well as CPC/PLGA$_{Sl}$ were found to be injectable. However, in combination with PLGA, formulations containing high Mw PVP showed an injectability of < 70 %. This is due to the fact that higher Mw PVP increases the viscosity of the liquid component [36], and when incorporated into CPC/PLGA results in pastes with a very high viscosity hampering extrusion from the syringe. It has been previously described that CPC pastes suffer from filter pressing, which means that when injected, the liquid phase of the CPC flows at a faster rate than the powder phase, leading to phase separation [27,38–40]. Here, CPC and CPC/PLGA formulations indeed suffered from this phase separation process, while PVP porogen incorporation diminished this effect. Evidently, the PVP acts as a lubricant and facilitates injectability.

As observed previously, cement cohesion was improved upon addition of PVP [24], with high Mw PVP showing excellent cohesion properties. CPC cohesion is of high importance as CPC leakage can induce an inflammatory reaction, blood clotting or pulmonary embolism, which can endanger the life of the patient [41–43]. While plain CPC paste showed bad cohesion due to a high fragmentation of the paste, CPC/PLGA showed fewer fragmentation. On the other hand, a large particulate cloud was formed, which has higher risk of leakage. Therefore, the enhancement of the cohesion in the PLGA-containing composites is of paramount importance. It has been described that paste viscosity can increase the cohesion of the CPC [44,45]. In view of this, PVPs with higher Mw generate solutions with higher viscosities that increase the cohesion of the paste. Others have incorporated carboxymethyl cellulose (CMC) into CPCs to improve their cohesive and lubricative properties [17,27,46–48]. Our study indicated that the inclusion of high Mw PVP performed as well as CMC. An additional advantage is that PVP Mw is not affected by gamma radiation sterilization [36]. This in contrast to CMC that is damaged by gamma radiation due to the breakage of the glycosidic bonds, which results in a reduced paste cohesion for CMC-containing CPC [27,49]. Consequently, PVP Mw might be an alternative to CMC as a cohesion promotor.

Several studies have investigated the *in vitro* degradation of CPC/PLGA composites and compared it to CPC [18,28,33,50–52]. In agreement with these studies, our data confirmed that the addition of PLGA enhances CPC degradation, which can be concluded from the higher mass loss, the lower pH and the increased calcium released into the incubation medium from PLGA-containing composites compared to the PLGA-free ones. Further, the data demonstrated that the *in vitro* degradation of CPC/PLGA composites did not occur during the first week

of incubation. In contrast, the addition of PVP porogens of any size and Mw resulted in a higher mass loss during the first week of incubation than CPC and CPC/PLGA composites. This increase in mass loss as such can be attributed to the PVP dissolution and coincides with the formation of macroporosity at an early stage. This initial mass loss occurred gradually and can prevent partial dissolution before setting, which would avoid CPC matrix densification.

The effect of pore size of bone substitutes on bone regeneration has already been extensively studied. However, limited agreement has been reached. While some suggest that the optimal pore size for bone ingrowth ranges from 80 – 400 μm [13,53,54], others have observed that pore sizes as small as 25 or 50 μm are sufficient for bone ingrowth [55–57]. In the current study, porogen sizes of ~50 and ~200 μm were selected. We observed that the mass loss using small PVP porogens was faster than using large PVP porogens, but the overall mass loss after 6 weeks was similar using both porogen sizes. This might be related to the fact that smaller particles have larger surface area to volume ratio, which speeds up the dissolution process. In contrast, the dissolution of the larger PVP particles occurs in a more gradual manner, which may be beneficial to avoid partial dissolution during setting. Feng et al. [58] studied the effect of pore size on *in vitro* degradation of CPC and observed that larger macropores enhanced CPC degradation due to an increase of the surface area. Further, Smith et al. [34] observed that the interconnectivity of the pores was enhanced with the addition of larger porogens. Therefore, the incorporation of large PVP porogens into CPC seems to be preferable. However, it has to be taken into account that this incorporation might be detrimental to the mechanical properties of the CPCs, as previously described [19,59].

It is known that the degradation rate of biodegradable polymers depends on the molecular weight, i.e. how lower the molecular weight, how faster the degradation [60]. In line with this, the effect of porogen Mw was investigated, which revealed that the mass loss in CPCs was enhanced at an early stage by the addition of low Mw PVP particles compared to high Mw particles. This early stage mass loss enhancement was also translated into an overall higher mass loss when combined with PLGA. This corroborates with previous studies, which showed that the dissolution rate of PVP was inversely proportional to its Mw [61–63]. It was concluded that this was due to the higher viscosity and hydrophobicity of high Mw PVP, and hence decreased wettability. To the best of our knowledge, PVP has never been incorporated into CPCs to accelerate degradation. Nevertheless, the effect of Mw of other polymeric porogens on CPC degradation has been studied. For instance, it was observed that PLGA porogens with low Mw enhanced faster CPC degradation *in vitro* [15]. Finally, it has been reported that low Mw PLGA porogens induced a superior bone formation compared with high Mw PLGA porogens, which was related to the faster degradation rate of low Mw PLGA [64].

5. Conclusion

The current study evaluated the inclusion of water-soluble PVP polymer into CPC and CPC/PLGA composites. Inclusion of PVP particles improved the injectability and cohesion of the CPC paste by increasing paste viscosity, but it also increased setting time. CPC and CPC/PLGA composites showed an increased early stage mass loss when PVP particles were incorporated. Low Mw weight PVP showed a faster dissolution rate than high Mw PVP. Particle size, on the other hand, had no large effect, but smaller-sized particles dissolved faster than larger ones. Overall, PVP Mw had a much stronger effect on handling properties and *in vitro* degradation than particle size. In conclusion, a correct fine-tuning of PVP particle size and, most importantly, Mw is of high importance to achieve CPC with excellent handling properties and degradation behavior.

Data availability

The authors declare the data supporting the findings of this work is available within the article. Extra data is available from the authors upon request.

CRediT authorship contribution statement

Irene Lodoso-Torrecilla: Methodology, Data curation, Writing - original draft, Visualization. **Floris Stumpel:** Data curation, Visualization. **John A. Jansen:** Conceptualization, Methodology, Writing - review & editing. **Jeroen J.J.P. van den Beucken:** Conceptualization, Methodology, Visualization, Writing - review & editing, Supervision, Validation.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

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