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Ready-to-use injectable calcium phosphate bone cement paste as drug carrier

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ABSTRACT

Current developments in calcium phosphate cement (CPC) technology concern the use of ready-to-use injectable cement pastes by dispersing the cement powder in a water-miscible solvent, such that, after injection into the physiological environment, setting of cements occurs by diffusion of water into the cement paste. It has also been demonstrated recently that the combination of a water-immiscible carrier liquid combined with suitable surfactants facilitates a discontinuous liquid exchange in CPC, enabling the cement setting reaction to take place. This paper reports on the use of these novel cement paste formulations as a controlled release system of antibiotics (gentamicin, vancomycin). Cement pastes were applied either as a one-component material, in which the solid drugs were physically dispersed, or as a two-component system, where the drugs were dissolved in an aqueous phase that was homogeneously mixed with the cement paste using a static mixing device during injection. Drug release profiles of both antibiotics from pre-mixed one- and two-component cements were characterized by an initial burst release of ~7–28%, followed by a typical square root of time release kinetic for vancomycin. Gentamicin release rates also decreased during the first days of the release study, but after ~1 week, the release rates were more or less constant over a period of several weeks. This anomalous release kinetic was attributed to participation of the sulfate counter ion in the cement setting reaction altering the drug solubility. The drug-loaded cement pastes showed high antimicrobial potency against *Staphylococcus aureus* in an agar diffusion test regime, while other cement properties such as mechanical performance or phase composition after setting were only marginally affected.

1. Introduction

Infection in bone (e.g., osteomyelitis) is one of the largest problems in orthopedic surgery, since it often results in a loss of bone tissue and the removal of implants in a second operation [1,2]. Owing to the limited accessibility of infected bone tissue to systemically administered drugs, a localized delivery of antibiotics is a common treatment of postoperative infections, e.g., using poly(-methyl methacrylate) (PMMA) beads as carriers for the drugs [3] or mixing self-setting PMMA cement with antibiotics [4]. However, PMMA-based materials are not resorbable [5] and require surgical removal, after which they may be replaced by either new material to prolong the antibiotic therapy or a permanent natural or synthetic bone graft. A significant step forward would be the use of degradable bone grafts impregnated with antibiotics, e.g., using sintered calcium phosphate phases [6] or self-setting calcium phosphate cements (CPC) [7–11]. In contrast to PMMA, this type of cement consists of a porous ceramic matrix, which is formed by a continuous dissolution–precipitation reaction after adding an aqueous phase to the cement powder. Depending on the pH value of the cement paste, two types of CPC can be distinguished: while at neutral and basic pH nanocrystalline hydroxyapatite is formed, a strong acidic pH by the addition of primary phosphates or phosphoric acid results in the formation of protonated secondary calcium phosphates such as brushite or monetite [12,13]. Since the set cement matrix is microporous, CPC have captured increasing attention for the controlled release of water-soluble drugs, such as antibiotics or bone growth factors [14].

CPC are commonly applied as powder/liquid formulations in which the cement powder is mixed during surgery with the aqueous cement liquid to produce the cement paste [15–17]. The paste is either modeled into an open defect by means of a spatula or it is (after transfer into a syringe) injected using minimally invasive operation techniques. The latter procedure exhibits intrinsic handling problems, since cement setting starts immediately after mixing the cement powder and liquid, resulting in a continuous change in the material properties and leaving only a small timeframe for cement application by the surgeon. Current developments in CPC technology concern the use of ready-to-use

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injectable cement pastes by dispersing the cement powder in a water-miscible solvent (e.g., glycerine [18,19], PEG [20]) such that, after injection into the physiological environment, setting of cements occurs by diffusion of water into the cement paste [21–23]. A new approach consists in the combination of a water-immiscible carrier liquid combined with suitable surfactants, which facilitates a discontinuous liquid exchange in CPC, enabling the cement setting reaction to take place [24,25].

The present study reports these novel cement paste formulations for use as a controlled-release system of antibiotics (gentamicin, vancomycin). Cement pastes were applied either as one-component material, in which the solid drugs were physically dispersed, or as a two-component system, where the drugs were dissolved in an aqueous phase, which was homogeneously mixed with the cement paste using a static mixing device during injection. Drug release kinetics were studied over a period of up to 56 days, and the influence of the admixed antibiotics on the material properties of the cement such as phase composition, mechanical performance or pore size distribution was determined.

2. Materials and methods

The cement powder composition used in this study was similar to the formulation of Biocement D, originally developed by Driessens and co-workers [26], and contained 60 wt.% α-tricalcium phosphate (α-$Ca_3(PO_4)_2$), 26 wt.% dicalcium phosphate anhydrous ($CaHPO_4$), 10 wt.% calcium carbonate ($CaCO_3$) and 4 wt.% precipitated hydroxyapatite. Tricalcium phosphate (TCP) powders were produced by sintering mixtures of $CaHPO_4$and $CaCO_3$in a 2:1 M ratio at temperatures of 1300 °C following quenching in air. The powder components were mixed in an agate ball mill (Pulverisette 5, Fritsch, Germany) with 30 g agate balls (Fritsch) at 200 rpm for 45 min. Mixing of the CPC powder and 4% Na_2HPO_4-solution with a powder to liquid ratio of 2.5 g ml^{-1} resulted in a water-based cement paste, which was used as reference material in the study. Ready-to-use cement pastes were obtained according to a previous study by Heinemann et al. [25]. Briefly, the CPC powder was mixed with 2.5% finely ground K_2HPO_4 in an oil-based suspension (synthetic short chain triglyceride Miglyol 812 with 8–12 °C saturated fatty acids) at an oil to powder ratio of 0.16 g ml^{-1}. The oil phase contained two surface-active agents, 14.7% (w/w) castor oil ethoxylate 35 (Cremophor ELP, BASF, Germany) and 4.9% (w/w) hexadecyl-phosphate (Cetyl-phosphate, Amphisol A, Brenntag AG, Germany). CPC powder and oil phase were mixed in a stainless steel mixer (Stephan Mischer, Stephan Machinery GmbH, Germany) until homogeneity. In accordance with standardized tests the cement paste was proved to be cyto compatible in vitro (DIN ISO 10993-5) and showed no sensitization and intracutaneous reactivity in animal studies (DIN ISO 10993-10) or systemic toxicity (DIN ISO 10993-11) [25]. The biocompatibility was demonstrated in an animal study (DIN ISO 10993-6) and showed reactions similar to a commercial CPC over a period of 90 days (data not shown) [25]. A study with human mesenchymal stem cells cultured on the used cement paste showed the ability of the cell to proliferate and differentiate into osteoblasts [27]. The cement pastes were applied as either one-component material or as a two-component system in which a second aqueous phase was homogeneously mixed with the cement paste at a 1:4 volume ratio using a static mixing device (Medmix, Switzerland) during injection (Fig. 1). Cement modification with gentamicin (molar mass, 463 g mol^{-1}; Fluka, Steinheim, Germany) or vancomycin (molar mass, 1486 g mol^{-1}; actavis, Munich, Germany) was performed (1) by mixing 1.24 or 2.48 wt.% solid antibiotic with the one-component cement paste during manufacture or (2) by dissolving 2.5, 5.0 or 10.0 wt.% antibiotic in the aqueous phase of the two-component cement system. Antibiotic-loaded reference cements were prepared by mixing the cement powder with a solution containing 10 wt.% gentamicin and 4 wt.% Na_2HPO_4 at a powder to liquid ratio of 2.5 g ml^{-1} or by adding 100 mg dry vancomycin to 2.5 g cement powder following mixing with 1 ml Na_2HPO_4 solution.

2.1. Release study

Cubic samples (6 × 6 × 12 mm) were fabricated in silicon molds and immersed in 3 ml of PBS buffer (composition: 8.0 g NaCl, 1.1 g Na_2HPO_4, 0.2 g KCl, 0.2 g KH_2PO_4) after 10 min setting. The release study was performed at 37 °C in an incubator with an orbital platform shaker (incubator 1000, Unimax 1010, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany), at a constant shaking rate of 60 rpm. The buffer was changed after each measurement. The vancomycin content of the eluate was determined directly by UV–vis spectroscopy at 237 nm. One milliliter of gentamicin containing eluates was mixed with 1 ml isopropanol and 1 ml of a solution containing 100 mg *o*-phthaldialdehyde, 1 ml methanol, 0.2 ml β-mercapthoethanol and 2 g sodium tetraborate in 100 ml water and measured after 45 min reaction time with UV–vis at

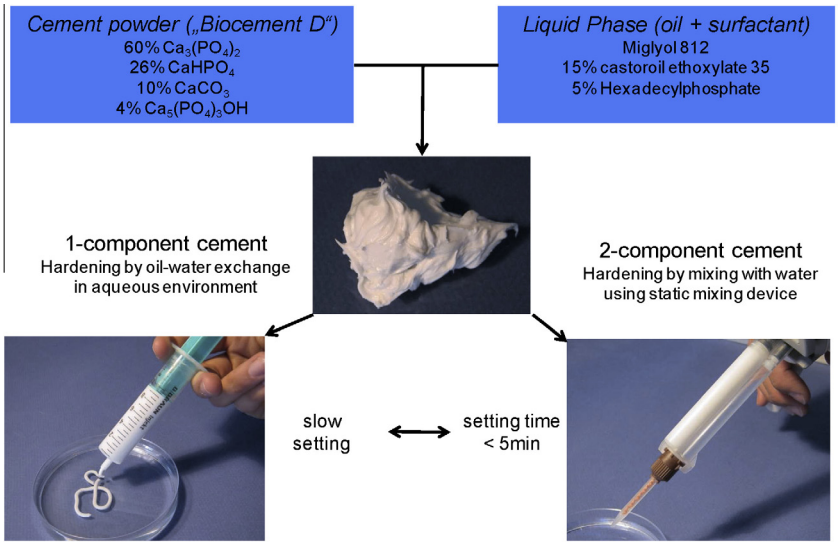

Fig. 1. Preparation regime of ready-to-use CPC pastes and their application in one- and two-component drug delivery devices.

332 nm. The total antibiotic content of the samples was determined after dissolution in 2 M HCl. The release profiles were fitted with the Korsmeyer–Peppas equation Eq. (2) up to 60% of the cumulative drug release.

2.2. Cement properties

Cubic samples 6 × 6 × 12 mm were fabricated for compression strength measurements and set for 3 days in PBS buffer solution. Mechanical testing was performed using a static mechanical testing machine (440, Zwick, Ulm, Germany) and a 5 kN load cell. Samples were loaded parallel to their long axis and were tested at a constant cross-head displacement rate of 1 mm min^{-1}. Average and standard deviation were calculated. Statistical analysis was performed using the Anova *t*-test in Microsoft Excel, in which drug-loaded cements were compared with unloaded cements for each cement system. Scanning electron microscopy (SEM; Zeiss DSM930) was used to analyse the gold-coated fracture surfaces of the ceramic monoliths. Samples were imaged using an accelerating voltage of 10 kV. X-ray diffraction (XRD) patterns of samples were recorded using monochromatic CuK_α radiation (D5005, Siemens, Karlsruhe, Germany). Data were collected from 2θ = 20–40° with a step size of 0.02° and a normalized count time of 1 s steps step^{-1}. The phase composition was checked by means of JCPDS reference patterns for α-TCP (PDF Ref. 09-0348), calcite (PDF Ref. 05-0586), hydroxyapatite (HA, PDF Ref. 09-0432) and monetite (PDF Ref. 09-0080). The calculation of the crystal size is based on the XRD analysis and the Scherrer equation Eq. (1) [28].

$$d = K\lambda/\beta\cos\theta \quad (1)$$

where λ = 1.541 Å is the X-ray wavelength of CuK_α radiation, θ is the diffraction angle, β is the full width at half maximum of the peak resulting from the crystallite size, and K is a constant related to the crystallite shape and, in this case, is equal to 0.9. Porosity characteristics such as pore size distribution, average pore size and pore volume were measured using a mercury porosimeter (PASCAL 140/440, Porotec GmbH, Hofheim, Germany). A contact angle of 141.3° and a surface tension of 480 mN mm^{-1} of mercury were used for calculation. The accuracy of measurement was ~1% for the detection of the volume and ~0.25% for the pressure. The errors for the pore size and the total porosity were than calculated by the maximum error law. The set cement samples were dried at 37 °C for 24 h prior to measurement. During the analysis, the pressure increased up to 400 kPa, followed by a faster pressure decrease to 0 kPa. The initial setting time of the two-component cement and reference cement with or without antibiotic loading was measured according to the Gillmore needle test at 37 °C and >90% air humidity with a needle of 113.98 g and 2.117 mm diameter according to the ASTM standard [29].

2.3. Antimicrobial tests

The antimicrobial activity of the samples was tested using the gram positive bacterium *Staphylococcus aureus*, strain RN 4220, in an agar diffusion test regime. The agar gel was prepared by dissolving 10 g Pepton/Trypton, 2 g yeast extract, 15 g agar and 5 g NaCl in 1000 ml water. The gel was autoclaved at 121 °C for 2 h prior to filling it into 100 mm petri dishes. The thickness of the gel was adjusted to 2 mm. Ten microliters of the bacteria was cultivated in 2 ml medium with an agar-free composition similar to that described for gel preparation for 24 h at 37 °C, diluted 1:4 with water, and 50 µl of this suspension was homogeneously dispersed on every agar plate. The preset cement samples (10 mm diameter, 5 mm height) were placed on the middle of the agar plates, and the inhibition zones around the cement samples were measured after 24 h cultivation at 37 °C. Average and standard deviation were calculated. Statistical analysis was performed using the ANOVA *t*-test in Microsoft Excel, in which drug-loaded cements were compared with unloaded cements.

3. Results

The release profile of both antibiotics from the pre-mixed one- and two-component cements were characterized by an initial burst release of ~7–11% (two-component; Fig. 2) or 15–28% (one-component; Fig. 3) and agree very well with the Peppas model [30]:

$$M_t/M_\infty = kt^n \quad (2)$$

Where M_t is the cumulative amount of released drug at time point t in milligrams, M_∞ is the initial load of drug in milligrams, M_t/M_∞ is the cumulative amount of drug released at time t in per cent, k (% s^{-1}) is the release constant, and n is the release exponent, which indicates the release process. Modeling of the drug release of one-component cements as well as gentamicin from the reference cement indicated a diffusion-controlled process (Figs. 3 and 4, n = 0.20–0.39; Table 1). The release of vancomycin and gentamicin from two-component cements and the release of vancomycin from the reference cement (Figs. 2 and 4) showed anomalous transport (n = 0.53–0.63) and hence was controlled by diffusion and the degradation process. After the initial burst, the vancomycin release followed a typical square root of time release kinetic, with a steady decrease in the amounts of drug released. Gentamicin release rates also decreased during the first days of the release study, but after ~1 week the release rates were more or less constant over a period of several weeks. The release kinetics from reference cements formed by mixing the cement powder with an aqueous phase (Fig. 4) demonstrated a slower release compared with the oil-based cement pastes with a much lower initial burst as well. After 56 days, 50% of gentamicin and 34% of vancomycin were released from the reference cement formulation, whereas the release of gentamicin could be categorized as a diffusion-controlled process, while vancomycin release was controlled by diffusion and degradation. During the complete release period of 56 days the pH-value of the release media was 7.38 ± 0.08 and therefore was always in the range of the physiological pH for all cement samples. The pharmacological activity of the released antibiotics was measured by the agar diffusion test, in which the samples were immersed in physiological buffer solution for up to 7 days prior to testing (Fig. 5). Both antibiotics were still highly active during this test, with visible inhibition zones of mostly >20 mm, which only marginally decreasing during the course of 7 days.

The initial compressive strength of set cements was found to be in the range of 10 MPa (two-component) and 18 MPa (one-component). While the addition of gentamicin had practically no influence on the mechanical performance of both cement types, even at high drug loads (Fig. 6), the addition of both 10 wt.% vancomycin to the cement liquid of the two-component paste and 2.48 wt.% directly to the cement of the one-component system significantly reduced the strength to ~7 MPa (two-component) and 13 MPa (one-component). In the two-component cement system, the storage under in vivo conditions led to an increase in the mechanical strength up to 23 MPa, while the mechanical strength of the one-component system was not changed after 56 days of immersion. Prior to complete setting in a liquid environment, the two-component system consisted of HA, monetite, calcite and unreacted α-TCP (Fig. 7). The immersion of the two-component cements finalized the setting reaction, and cements were composed of a low crystalline hydroxyapatite phase according to XRD analysis, with a minor amount of monetite (Fig. 7). Although no influence of drug modification on the degree of conversion to HA was detected by

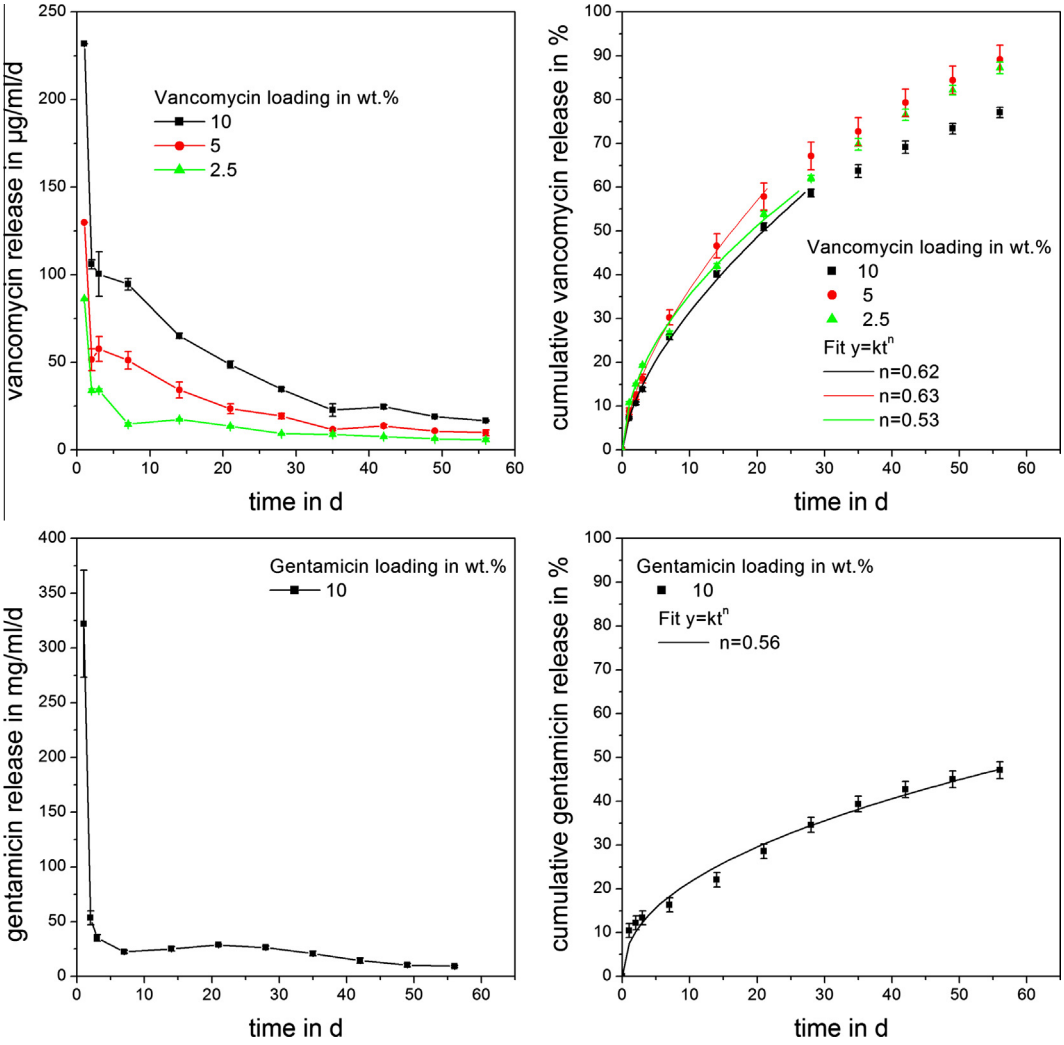

Fig. 2. Drug release from two-component ready-to-use cement pastes into PBS buffer, depending on drug type and load.

XRD, the addition of vancomycin increased the crystal size from ~29 to 35 nm (Fig. 7a). Furthermore, the SEM analysis shows an alteration of the microstructure by adding the antibiotics. While the drug-free reference cement exhibited a typical microporous plate-like morphology, both gentamicin and vancomycin led to cement structures composed of spherical crystal agglomerates in the sub-micrometer size range (Fig. 8). The pore size distributions of the cements showed large differences regarding the type of cement application and a minor reduction in the pore size due to drug modification (Table 2). However, the pore size distributions were independent of the immersion time, regarding each system separately. The reference cement prepared by directly mixing cement powder with the aqueous sodium phosphate solution was characterized by small pores (4–100 nm) with an average pore size of 15.4 nm and the absence of larger pore sizes. Moreover, the premixed pastes exhibited larger pore sizes up to 1 µm (two-component) and ~100 µm (one-component). Total porosities were found to be in a size range of ~42% (reference powder/liquid cement), 19% (one-component pre-mixed) and 21% (two-component pre-mixed) (Fig. 9, Table 2) for the unloaded cements. Owing to experimental deviations in the case of cement preparation, total porosities were proved to be the same for unloaded and drug-loaded cements, for one- or two-component cements as well as in an initial state of setting or after 3 days of immersion.

Table 3 shows the cement setting time of the reference cement and two-component system. All samples were hard enough to be handled after 10 min and could be removed from the molds for the release experiments, although a complete setting reaction could not be presumed at this time point. In particular, one-component cement samples only set under liquid conditions, since the paste was not actively mixed with a binder solution, and the hydraulic reaction is triggered by a water–oil exchange in a wet environment.

4. Discussion

The gold standard treatment for the prevention or treatment of bone infections (osteomyelitis) mainly caused by *S. aureus* are PMMA [31] beads or cements loaded with antibiotics, such as vancomycin [32] or gentamicin [33] as an adjunct to surgical treatment. However, PMMA-based cements are only marginally porous, and diffusion of loaded antibiotics into the surrounding bone tissue is limited to the outer surface of the cement, such that only a minor amount of the incorporated drug (<15%) is released, even after a long time [34]. In contrast, mineral cements based on CPC chemistry are microporous, with an aqueous phase filling the porous structure. The potential of CPC as drug delivery devices has been reviewed recently [14], showing good compatibility and sufficient release of many different drug types, such as antibiotics, proteins and anti-inflammatory drugs, from CPC matrices. Technically, the drugs are either dissolved in the cement liquid or they are physically mixed with the cement powder in dry particulate form,

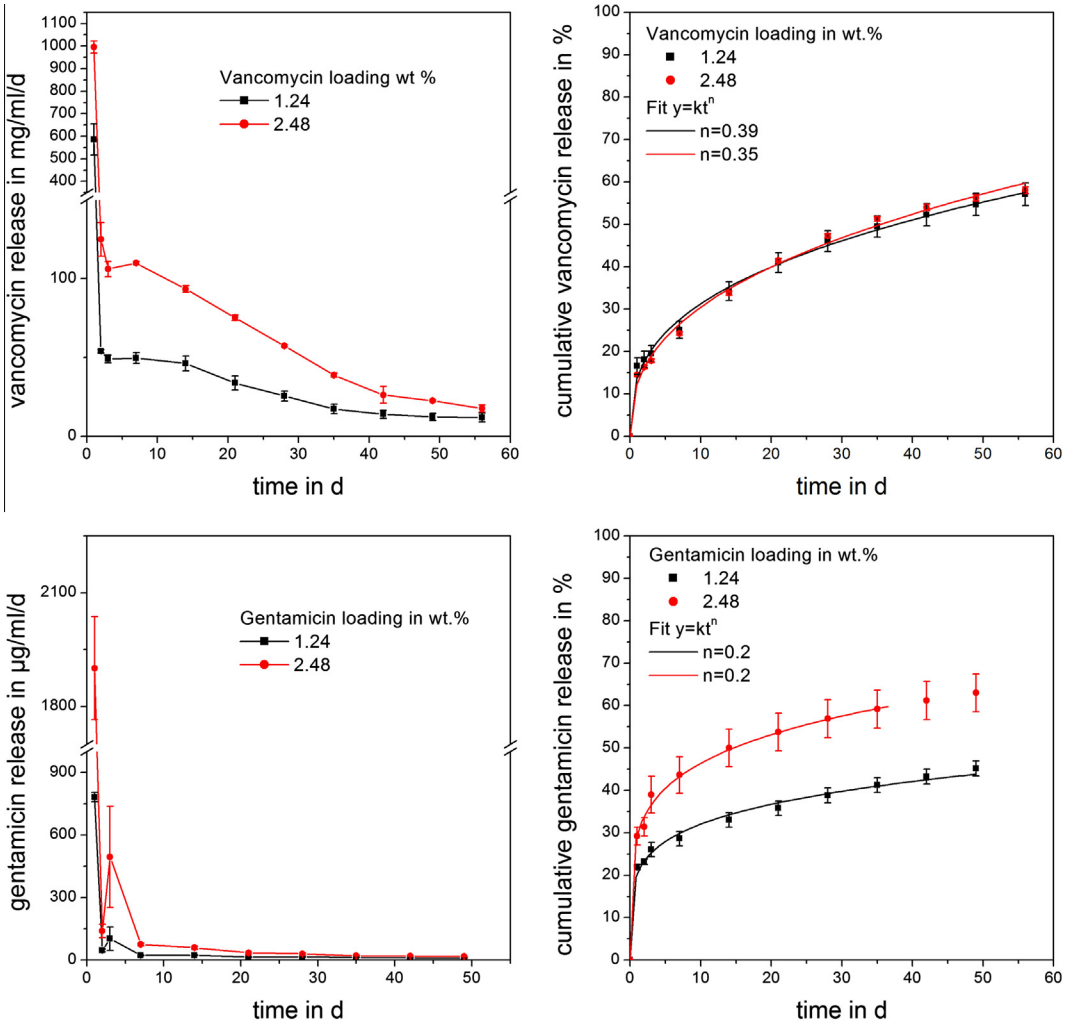

Fig. 3. Drug release from one-component cement pastes in PBS buffer, depending on drug type and load.

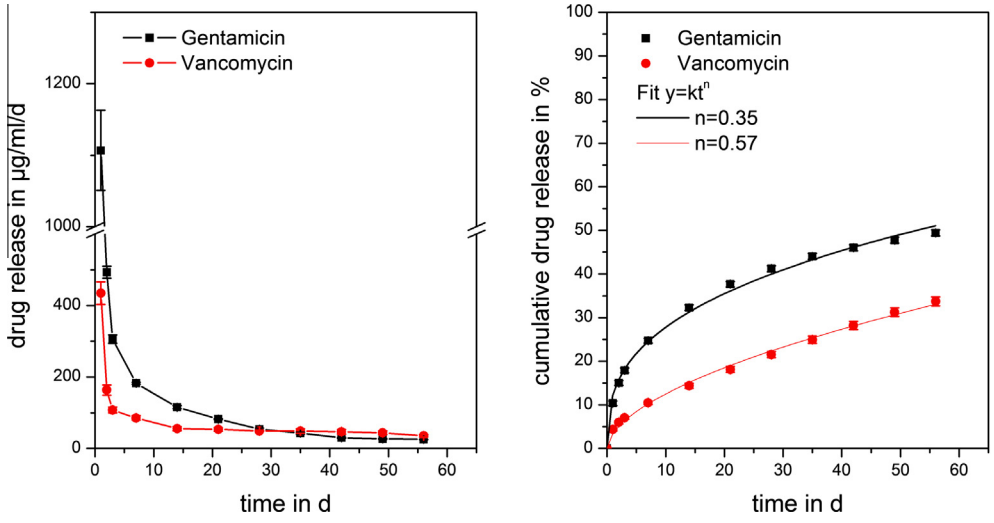

Fig. 4. Drug release from powder/liquid cement pastes in PBS buffer, depending on drug type.

following mixing of cement liquid and powder to obtain the cement paste. Similar approaches were made in the current study for the drug modification of pre-mixed CPC pastes based on cement–oil mixtures (Fig. 1). On the one hand, the antibiotics used were dispersed in solid form in the cement–oil paste during preparation, leading to a one-component cement paste, which hardens by water–oil exchange in a wet environment (followed by the hydraulic setting reaction) [25]. On the other hand, the drugs were

Table 1
Fitting parameters of the release profiles according to the Peppas model with the release controlling processes.

Cement	Antibiotics	Fit parameters		Release process controlled by
		k (%)	n	
Two-component	10% Vancomycin	7.50 ± 0.44	0.62 ± 0.02	Diffusion/degradation
Two-component	5% Vancomycin	8.57 ± 0.47	0.63 ± 0.02	Diffusion/degradation
Two-component	2.5% Vancomycin	10.43 ± 0.40	0.53 ± 0.01	Diffusion/degradation
Two-component	10% Gentamicin	7.51 ± 0.72	0.46 ± 0.03	Diffusion/degradation
One-component	2.48 wt.% Vancomycin	12.39 ± 0.61	0.39 ± 0.01	Diffusion
One-component	1.24 wt.% Vancomycin	13.84 ± 0.59	0.35 ± 0.01	Diffusion
One-component	2.48 wt.% Gentamicin	29.57 ± 0.65	0.20 ± 0.01	Diffusion
One-component	1.24 wt.% Gentamicin	20.37 ± 0.59	0.20 ± 0.01	Diffusion
Reference	1.24 wt.% Vancomycin	3.41 ± 0.24	0.57 ± 0.02	Diffusion/degradation
Reference	1.24 wt.% Gentamicin	12.33 ± 0.55	0.35 ± 0.01	Diffusion

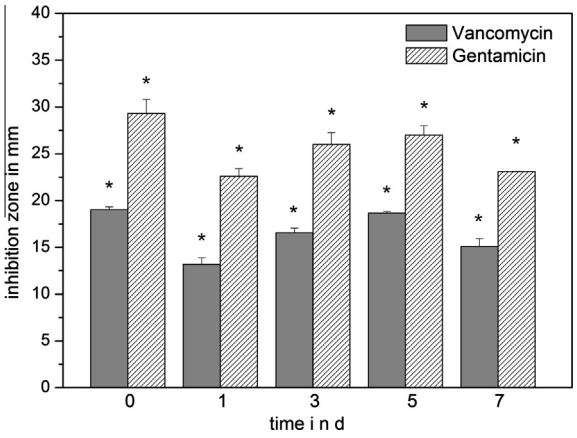

Fig. 5. Medium inhibition zones in agar diffusion test of two-component preset-cement samples against *S. aureus* after elution the cement samples in PBS for up to 7 days with a change of immersion medium after each time point. The two-component cement was used for this test, since it enabled sample preparation without immersion in water, such that no loss of antibiotics occurred during setting for $t = 0$ days. The antibacterial activity was significantly higher compared with unloaded cement ($P < 0.05$).

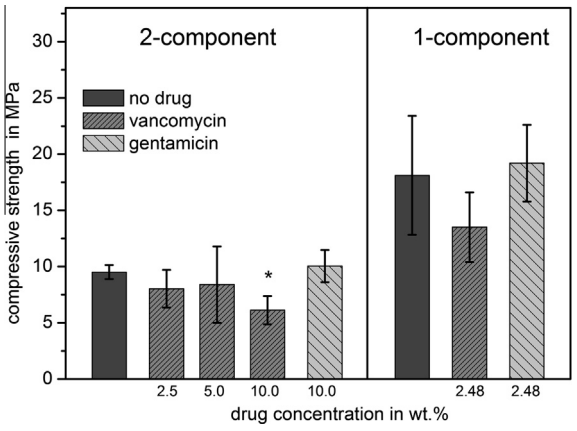

Fig. 6. Compressive strength of antibiotic loaded cements after 3 days' setting at 37 °C. The mechanical strength of two-component cement was significant reduced by adding 10% vancomycin with $^{*}P = 0.012$.

dissolved in a small amount of water, and this solution was then actively mixed with the cement–oil paste, using a static mixing device during injection. Both methods have certain (dis)advantages regarding the ease of use, choice of drug type and concentration, and the setting characteristics (Table 4).

The drug release kinetics was influenced by both the type of drug and the form of cement application. In all cases, the release of vancomycin followed a first-order kinetic with a total release of ~33% (reference), 58% (one-component) and 77–89% (two-component) after 56 days' elution and agreed very well with the Peppas model. Similar release profiles of vancomycin were also found in other studies [35–37], independent of the cement chemistry, whereas only the total amount of drug released was different. Most likely, the release of this drug from the cement matrices is influenced by physical cement properties (e.g., porosity, pore-size distribution) as well as by the cement setting reaction, rather than by chemical interactions between the drug or cement ingredients. The analysis of the release profile with the Peppas model indicated a process controlled by diffusion for one-component cements as well as for the gentamicin-loaded cement produced as reference. The complete hardening of the one-component system occurred within the first day of immersion by water–oil exchange and was accompanied by turning of the cement from a pasty to a hard state. Therefore, the release of drugs was diffusion controlled, with a high initial burst release during the pasty state, decreasing with the proceeding setting reaction, and a corresponding decrease in the diffusion coefficient within the cement volume material and an increase in porosity. Since cement samples of two-component system and reference cement were set prior to immersion, the porosity was presumed to be the determining factor of the release kinetic. Here, the pore size as well as the tortuosity of the pores determined the diffusion process, which did not change during the release time. Furthermore, the pore size distribution of the cements seemed to be a more critical parameter than total porosity in the current study. The water-based reference cement showed the slowest drug release with the highest total cement porosity of 42%, which is probably a result of the very small pores in a size range of 4–100 nm with 97% pores <10 nm. Here, the reason for the slower release of vancomycin compared with gentamicin might be the higher molar mass of vancomycin (molar mass = 1486 g mol^{-1}) compared with gentamicin (molar mass = 463 g mol^{-1}), which involved a higher diffusion coefficient. In contrast, both pre-mixed cement–oil pastes provided a faster release at lower porosities as a consequence of larger pores in the sub-micrometer size range, with more than 28% of pores >10 nm and a mean pore size of 18–30 nm (Table 2). These larger pores are formed either by the dispersion of the aqueous (drug containing) liquid in the cement using the static mixing device (two-component cements) or in situ during release, by the dissolution of the solid antibiotics dispersed in the one-component paste.

In contrast, cement modification with gentamicin (sulfate) was more susceptible to the type of cement regarding the release kinetics. Although the total amount of released gentamicin after 56 days' immersion in PBS buffer was found to be within a narrow range of 45–63% (smaller molecule than vancomycin, less susceptible to pore size), the typical first-order release kinetics from the water-based reference cement (Fig. 4) changed to a three-phase profile with (1) an initial burst followed by (2) a zero-order release

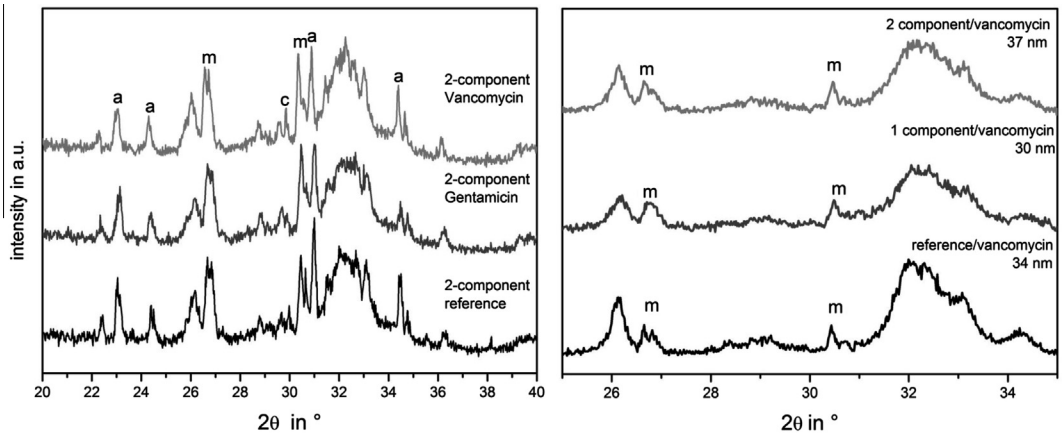

Fig. 7. XRD patterns of (a) antibiotic modified two-component cement pastes after 3 days' setting at 37 °C and (b) different cements loaded with vancomycin at the end of the release study (56 days) with the crystal size of HA. The diffraction patterns correspond to low crystalline hydroxyapatite with minor phases of: a, α-TCP; m, monetite; c, calcite.

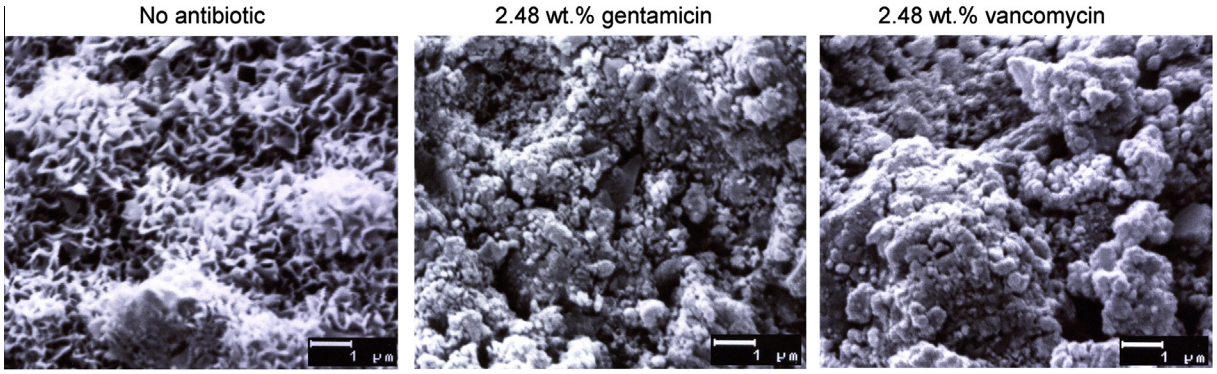

Fig. 8. Microstructure of cements (one-component) after 3 days' setting at 37 °C.

Table 2
Characteristic values of the porosity according to the cement preparation method and drug modification.

Cement/antibiotic	Total porosity (%)	Average diameter (μm)
Reference/–	42 ± 2	0.0154 ± 0.0002
One-component/–	19 ± 2	0.0182 ± 0.0002
Two-component/–	21 ± 2	0.0301 ± 0.0003
Two-component/10% gentamicin	18 ± 2	0.0239 ± 0.0003
Two-component/10% vancomycin	20 ± 2	0.0249 ± 0.0003

The errors were calculated by the maximum error law, depending on the accuracy of measurement.

kinetic up to day 30–35 and (3) a decrease in the released drug doses afterwards. This behavior was found especially in the two-component cements in which the gentamicin was dissolved in water and then homogeneously mixed with the cement–oil paste. A reason for this is probably interference of the gentamicin sulfate with the anionic hexadecyl-phosphate surfactant within the pastes, which may alter the solubility of the drug. This effect may be more pronounced for the anionic gentamicin molecule than for vancomycin with less positive charge density, since the sulfate counter ion is more able to take part in the cement setting reaction (precipitation as calcium sulfate) rather than the chloride ion from vancomycin hydrochloride. In addition to the hexadacyl-phosphate, the calcium phosphate matrix itself my act as an ion exchanger in which calcium can be replaced by gentamicin to some extent, thus contributing to the extended release profile. However, these interactions do not influence the pharmacological properties of the released antibiotics. Both drugs showed a high antimicrobial activity against *S. aureus* even after an immersion time of 7 days (Fig. 5).

Setting of mineral biocements occurs by a continuous dissolution–precipitation reaction. In particular, the setting reaction of HA-forming cements is known to be susceptible to inorganic or organic additives, which may act as HA crystal growth inhibitors. It is known that the addition of magnesium or pyrophosphate ions [38,39] or drugs such as gentamicin crobefate [8] can inhibit HA growth and hence cement setting. Although the addition of both antibiotics (including other cement ingredients such as oil and surfactants) from the present study changed the cement microstructure (Fig. 8), no influence was found on the phase composition (Fig. 7). A marginal effect was found for the mechanical performance (Fig. 6), in which higher concentrations of vancomycin slightly decreased the compressive strength, probably as a result

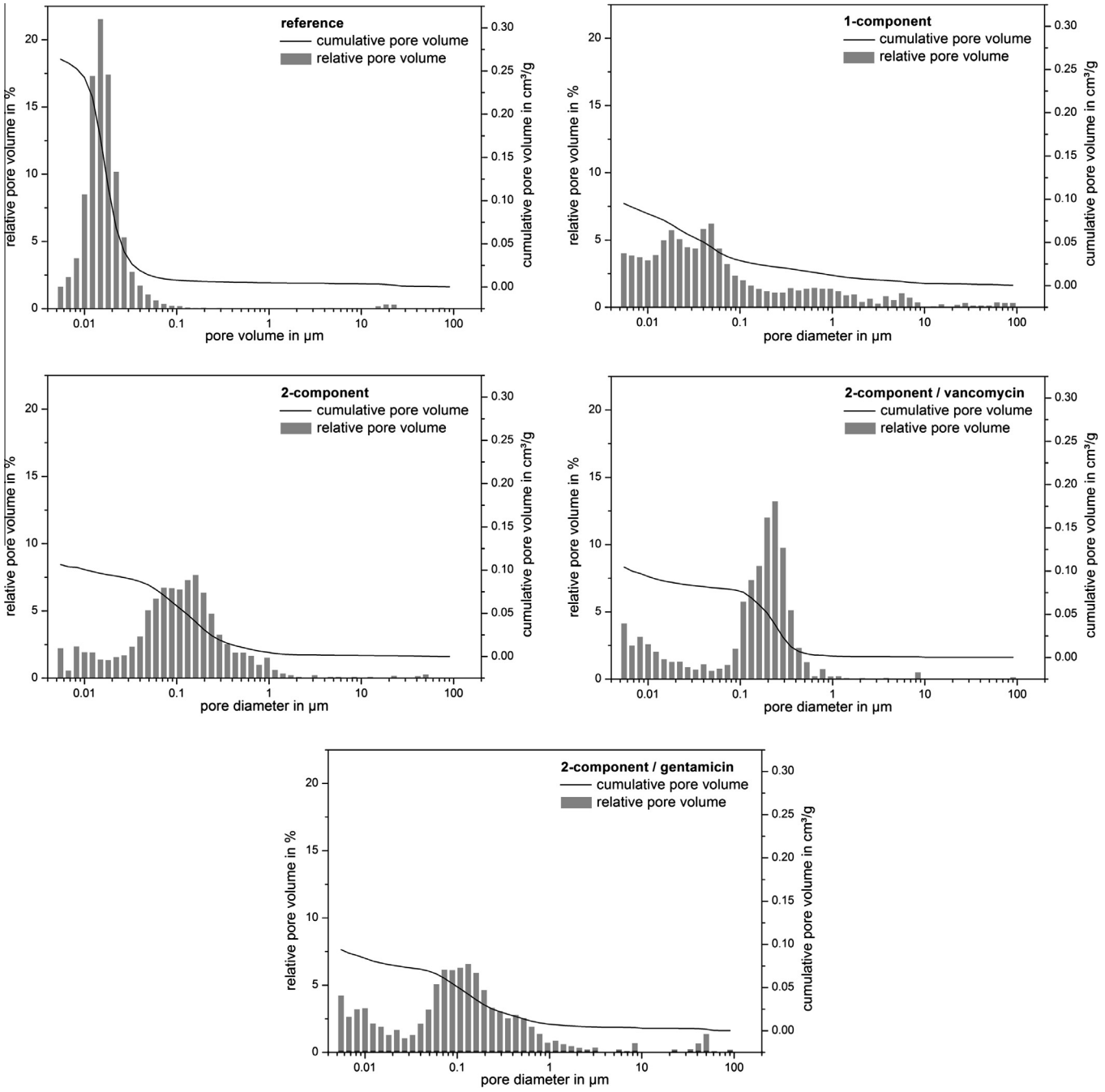

Fig. 9. Pore size distributions of CPC used in this study.

Table 3
Setting time of pure and drug-modified cements for the different application methods.

Cement	Antibiotic	Setting time (min)
Two-component	–	3.6 ± 0.1
Two-component	10% Vancomycin	8.4 ± 0.3
Two-component	10% Gentamicin	5.7 ± 0.3
Reference	–	9.7 ± 0.6
Reference	10% Vancomycin	9.8 ± 1.1
Reference	10% Gentamicin	9.5 ± 0.5

of increasing crystal size, whereas gentamicin had no significant effect, even at high concentrations. The principal difference of the one- and two-component pre-mixed cement pastes regarding their strength is related to the different phase composition in the initial stage of both application types and to the differences in the porosity. Although porosity increases only from 19% (one-component) to 21% (two-component) by the dispersion of an aqueous solution in the cement–oil paste in a 4:1 volume ratio, strength was found to decrease simultaneously by a factor of 2.5, owing to the well-known inverse exponential relationship between both values in porous bioceramic matrices [40]. Moreover, with increasing immersion time, the unreacted α-TCP in the two-component cements transformed into HA, which resulted in strengthening from 10 to 22 MPa for the unloaded cement (Figs. 7 and 6). The observed differences in compression strength compared with recently published data with comparable cement preparations [18] are attributed to modified testing protocols: 3 vs. 4 days' setting period

Table 4
Advantages (+) and disadvantages (−) of using one- and two-component pre-mixed cement pastes as drug delivery devices.

One-component cements	Two-component cements
+ Higher strength due to lower cement porosity	+ Free choice of drug type and concentration by customer
+ Easier to handle, interruption of cement injection possible	+ Rapid hardening in volume
− Slow hardening in volume	− Continuous injection necessary*
− Fixed drug type and concentration	− Lower mechanical performance

* Otherwise the static mixer has to be replaced.

and silicone molds instead of stainless steel molds. It was observed that the more precise and rigid steel molds generally result in testing samples with fewer flaws and thus show considerably higher compression strength values (for powder/liquid cements as well as for paste cements) than samples prepared in silicone molds, although there was no difference in sample preparation otherwise.

Clinical applications of the drug-modified pre-mixed CPC might include the treatment of osteomyelitis, in a similar way to PMMA-based cement, with the advantage of a higher antibiotic release rate. In contrast to PMMA, the mineral cements can remain at the application site after drug release and serve as bone grafting materials, since they are known to be osteoconductive [41], and they can be resorbed by osteoclastic activity [42]. Another attractive application concerns the prevention of post-operative bone infections during implantation of metallic prosthesis (e.g., hip/knee or osteosynthetic devices) in order to provide localized antibiotic protection to non-cemented implants and filling bone defects at the same time. In particular, the one-component pastes from the present study may be used to apply an antimicrobial cement coating onto the implants, which delivers the antibiotic to the most endangered site—the bone–implant interface—during surgery. In contrast to commercially available coatings on endoprosthesis which are commonly based on thin polymer coatings, this method would have the advantage of using only biodegradable materials, so that the host bone can still grow directly onto the implant surface without creating additional interfaces (polymer in contact with implant or polymer surface in contact with bone). The two-component system may be considered for the targeted treatment of local bone infections in combination with bone defect filling, as this system provides the opportunity to choose an appropriate antibiotic combination fitting the susceptibility profile of the infecting pathogen. In both cases, the carrier systems described and the selected antibiotics—gentamicin and vancomycin—show long-term release profiles that compare favorably with established PMMA-based antibiotic carriers. Further studies will be directed towards additional appropriate antibiotics for the local treatment of bone infections.

5. Conclusion

The present study demonstrated the effectiveness of pre-mixed CPC-oil pastes as carriers of antibiotics for the treatment of osteomyelitis, since the release rate exceeded the minimum inhibitory concentration for most pathogens over a long period. Furthermore, the amount of released drug can be controlled by the antibiotic concentration in the cement paste. The cartridge system provides user-optimized handling properties for this HA cement, with the opportunity for targeted treatment of localized bone infections.

Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figs. 1–4, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at 10.1016/j.actbio.2013.08.009.

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