RIGA TECHNICAL UNIVERSITY
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INFLUENCE OF COMPOSITION OF α-TRICALCIUM PHOSPHATE BASED BONE CEMENTS ON THEIR STRUCTURE AND PROPERTIES

Summary of Doctoral Thesis

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CONFIRMATION

I confirm that I have developed the present Doctoral Thesis, which is submitted for consideration at Riga Technical University for scientific degree of the doctor of engineering sciences. The Doctoral Thesis has not been submitted at any other university for the acquisition of a scientific degree.

Zilgma Irbe.....(Signature)

Date:.....

The Doctoral Thesis is written in Latvian language; it contains Introduction, 3 chapters – Review of Literature, Methods, Discussion of Experiments, Conclusions, list of References, as well as 61 illustrations and 25 tables, altogether 131 pages. 165 references are used for this Doctoral Thesis.

ACKNOWLEDGMENTS

I would like to thank my scientific supervisor Prof., *Dr. sc. ing.* Līga Bērziņa-Cimdiņa and colleagues *Dr. sc. ing.* Jānis Ločs and *Dr. sc. ing.* Dagnija Loča, as well as to all employees of Institute of General Chemical Engineering for help and advice during writing of this work.

Most sincere thanks to my family for support, understanding and encouragement.

TABLE OF CONTENTS

OVERVIEW OF THE DOCTORAL THESIS

Current situation and actuality

Musculoskeletal diseases are some of the most common reasons for a person's inability to work in both developed and undeveloped countries, especially after 50 years of age. It is predicted that number of persons suffering from musculoskeletal diseases will rise significantly over next two decades [1, 2]. Surgery is often required for treatment of musculoskeletal diseases and many skeletal pathologies are treated using various implant devices. Therefore it can be foreseen that demand for quality biomaterials for treatment of bone and joints will rise. Research on bone substitute materials is of significant importance to advance treatment.

Calcium phosphate biomaterials (ceramics, cements, glasses and glass ceramics) are applied to treat and augment bone defects in non-load bearing applications. Calcium phosphate materials are generally considered bioactive and osteoconductive and can be bioresorbable [3].

Calcium phosphate biomaterials in general have weak mechanical properties; therefore they are used in non-load bearing applications, such as maxillofacial surgery.

Calcium phosphate (and also other calcium salt) cements have attracted attention and research activities of many scientist groups. Unlike ceramics, glasses and many polymer and metallic biomaterials, calcium phosphate cements are formed during implantation – this way calcium phosphate cements can be made to exactly fit the bone defect. Calcium phosphates set after implantation, are biocompatible and biodegradable.

Calcium phosphate cements are also excellent drug carriers. Drugs and other biologically active substances can be incorporated directly or as capsules into the setting cement paste.

Though a large amount of research has been conducted about calcium phosphate cements, there is still much needed to improve

mechanical properties, cement setting, cohesion and biological properties.

Calcium phosphate cements are implanted during surgical operations, therefore it is of great importance that cement can be sterilized and that it is easy to use – simple and quick mixing, high injectability (if needed), good cohesion and quick setting time (5 - 15 min).

The aim of the Doctoral Thesis

After analysis of literature, a following aim was set – to develop calcium phosphate bone cement compositions with controllable properties, including setting time, injectability, and cohesion.

Tasks set to realize aim of the Doctoral Thesis:

- assess possibilities and techniques that can be used to controllably influence properties of calcium phosphate bone cements, by changing cement composition,
- assess the influence of cement composition on mechanical strength, morphology and phase composition of set cement,
- evaluate interaction between developed calcium phosphate cements and living cells and tissue, using *in vitro* and *in vivo* tests,
- select the most suitable cement compositions for practical application.

Scientific significance of the Doctoral Thesis:

- for the first time the relations between properties and composition of α-tricalcium phosphate based cements have been comprehensively evaluated,
- the influence of composition and structure on release kinetics of local anesthetic drug (lidocaine) for α-tricalcium phosphate based cements has been evaluated.

Practical significance of the Doctoral Thesis:

- a method to control setting time of α-tricalcium phosphate based cements using the composition of cement liquid phase developed,
- a comprehensive set of procedures has been developed to assess properties of α-tricalcium phosphate based cements.

Scientific novelty

For the first time the influence of cement liquid phase composition on calcium phosphate bone cement (based on α-tricalcium phosphate) properties and processes during cement setting and aging has been systematically researched. As a result unique calcium phosphate cement compositions have been developed.

Statement to defend

By changing the composition of cement liquid phase, it is possible to modify calcium phosphate cement properties in a controllable and desirable way.

Approbation and publication of the Doctoral Thesis

The results of scientific research for this thesis have been approbated in 4 full text publications and 10 short conference proceedings. The results have been presented in 11 international scientific conferences. 1 patent has been applied for in the Republic of Latvia.

REVIEW OF LITERATURE

Calcium phosphates and calcium phosphate cements are widely researched as biomaterials for bone augmentation and regeneration. For the application of calcium phosphate cement the ease of use (including appropriate setting time and good cohesion) is of utmost importance. The setting of calcium phosphate cements starts immediately after mixing of the cement paste and cement is set after a few minutes.

The precipitation of calcium phosphates from aqueous solutions (the reason for cement setting) is influenced by two main parameters – temperature and the composition of the solution. The setting of the calcium phosphate cements is influenced by several factors – the composition of the solid phase, powder/liquid ratio, particle size and also by the two aforementioned factors.

The methods for determining calcium phosphate bone cement properties (for example, setting time, biodegradation speed, cohesion) have no set standards.

There are several kinds of calcium phosphate cements and their biodegradation speed can differ significantly. Cements that are composed of stoichiometric hydroxyapatite after setting are biodegraded very slowly. Cements that are composed of non-stoichiometric hydroxyapatite are biodegraded faster. Cements that are composed of dicalcium phosphate dihydrate or brushite are biodegraded fast (more than 80% of the cement in a year).

Several commercial calcium phosphate bone cements are available on the market; calcium phosphate cements that form hydroxyapatite after setting have the most of commercial demand.

On the basis of literature review, α-tricalcium phosphate (α-TCP) was chosen as a starting material for calcium phosphate cements developed in this doctoral work. It is comparatively easy to obtain and it is possible to obtain cement formulations that induce little chemical irritation (compared to brushite cements). Cements based on α-TCP have moderate biodegradation speed, are biocompatible and osteoconductive.

METHODS

The starting material of the investigated cements is α-tricalcium phosphate (α-TCP). In this work α-TCP is obtained by *high-temperature synthesis* – mixture of calcium carbonate and calcium hydrogen phosphate dihydrate (DCPD or mineral brushite) with molar ratio 1:2 is thermally treated at 1300°C or 1400°C. The synthesized α-TCP is mechanically activated (increased reactivity) by milling in a planetary ball mill. The amount of magnesium in starting materials and synthesis products was determined using *X-ray fluorescence method (XRF)*.

Sodium phosphate solutions with various pH values and setting time modifiers (citrate, tartrate and pyrophosphate salts) are used as a liquid phase.

The crystalline phases of starting materials and cement samples were determined using *X-ray diffraction method (XRD)*. The morphology was determined using *field emission scanning electron microscopy (SEM)*.

Reactions in cement samples (if less than 24 h old) prepared for XRD and SEM were stopped using two methods: by suspending cement particles in ethanol and drying afterwards in air (a) and by freezing samples in liquid nitrogen and freeze-drying afterwards (b). The aging of the cement samples was done in physiological saline at 37°C.

The setting time was determined using modified ISO 9917-1:2007 standard method. On the surface of the cement a perpendicular needle weighting 270 g with 1 mm diameter was placed and released. When needle did not leave a complete indentation on the surface, cement was considered set.

The injectability of the cement was evaluated by comparing liquid/solid ratio of the injected paste and of the paste before injection, as well as by amount of paste that could be injected before the syringe was blocked.

The cohesion of the cement was evaluated by placing not yet set cement paste (2 min after mixing) in physiological saline at 32°C. After 24 h, the amount of particles that had fallen from the cement surface was weighted. The fallen particles were also visually examined.

The release of lidocaine (local anesthetic drug) from cement matrices was evaluated using *high-performance liquid chromatography*.

In vitro tests were done using MG63-GFP cells (human osteoblasts), The criteria used were: the amount of cells attached and proliferating on the

cement surface and the morphology of the cells. *In vivo* tests were conducted by implanting cement samples in form of not yet set paste in rabbit (breed '*Californian'*) lower jaw and femur defects. The augmented bone was explanted after 3 months.

DISCUSSION OF EXPERIMENTS

Synthesis of α-tricalcium phosphate

α-TCP is the high temperature form of β-TCP. Transformation temperature of β-TCP to α-TCP (as described in literature [4]) happens at 1125°C. By quenching in air it is possible to obtain α-TCP at room temperature.

All α-TCP synthesis had a β-TCP (β-tricalcium phosphate) admixture. It was not possible to obtain completely pure α-TCP by heating the synthesis to 1400°C and quenching. It was also not possible to obtain pure β-TCP if synthesis was slowly cooled from 1300°C (together with the furnace).

A long synthesis time (more than 1 h) at temperature that is higher than transformation temperature from β-TCP to α-TCP, is not necessary as long holding times do not result in significantly altered α-TCP content, see Fig. 1.

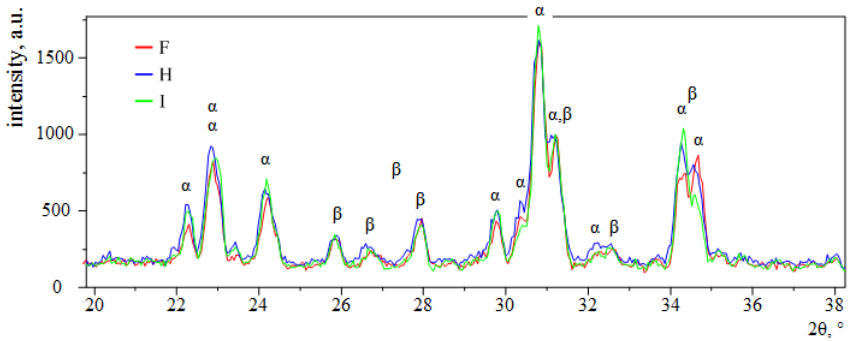

Fig. 1. XRD patterns for syntheses F (5 h at 1300°C), H (4 h at 1300°C and 1 h at 1400°C) and I (1 h at 1400°C), α-TCP marked as 'α', β-TCP as 'β'

The speed of quenching does not affect the amount of β-TCP admixture in α-TCP significantly.

It is described in literature [4, 5] that presence of magnesium can increase β-TCP to α-TCP transformation temperature.

In the presence of magnesium (0,15% at least in the conducted syntheses) a partial transformation from α-TCP to β-TCP happens. The reason for this partial transformation can be the exclusion of magnesium from α-TCP structure as it forms. As a result the rest of mass has higher magnesium content than newly formed α-TCP. As the untransformed β-TCP has more magnesium than original mass had and the transformation temperature rises.

The setting of α-tricalcium phosphate based cements

The setting time of cements is dependent on initial liquid phase pH, see Fig.2.

The fastest setting occurs if initial liquid phase pH is close to neutral. If the initial liquid phase pH is more basic or more acidic, the setting time increases.

If setting temperature is increased from room temperature to body temperature (from 21°C to 37°C), setting time decreases significantly for all cements investigated, see Fig.2. The reason for this is the faster reactions in cement at higher temperatures. This is a positive factor as before implantation cement paste is liquid/moldable, but after implantation will set quickly.

The presence of cement setting modifying substances (additives) – citrate, tartrate and pyrophosphate ions – can increase or decrease setting time depending on initial liquid phase pH, see Fig. 3 (with citrate ions).

The addition of citrate ions to cement liquid phase changes the curvature of setting time graph. After adding citrate ions the fastest setting time is at acidic initial liquid phase pH values (6 – 6,5), not at pH 7.

In literature [6] it is shown that α-TCP is more soluble at acidic pH values, therefore more new phases can be precipitated. Data in Fig. 3 show that reaction is faster at more acidic initial liquid phase pH.

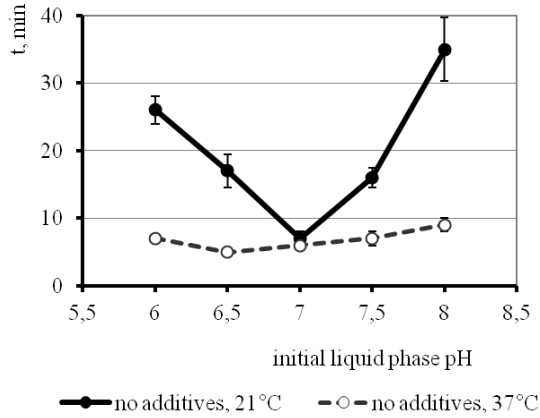

Fig. 2. Setting time depending on initial liquid phase pH at 21°C and 37°C temperature, powder/liquid ratio 1,75 g/ml, 0,5 M phosphate ions in cement liquid phase

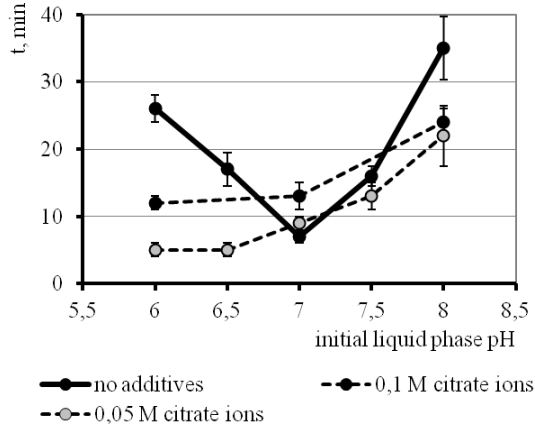

Fig. 3. Setting time depending on pH and amount of citrate ions in the liquid phase and initial liquid phase pH, at 21°C temperature, powder/liquid ratio 1,75 g/ml, 0,5 M phosphate ions in cement liquid phase

If cement liquid phase additives are not used and acidic initial liquid phase pH is used, during cement mixing a fast precipitation reaction occurs (precipitation of calcium hydrogenphosphate dihydrate, mineral name brushite, further in this text abbreviated as DCPD) and the further cement setting reactions are delayed. As a result setting at acidic liquid phase pH is slower than at neutral pH.

In literature [7] it is described that citrate ion additives delay setting reactions for cements that form brushite during setting.

In cement liquid phase is acidic and contains citrate ions, DCPD precipitation does not occur and the setting time for this composition is faster than for cement composition without citrate ions.

The setting time graphs are similar for cements containing 0,1 M and 0,05 M citrate ions in the liquid phase. Setting time (at the same initial liquid phase pH values) is longer for cements containing 0,1 M citrate ions. This suggests that presence of citrate ions also inhibits cement setting.

If tartrate ions are added to liquid phase, the fastest setting occurs at initial liquid phase pH 7. Tatrate ion addition prolongs setting at tasted initial liquid phase pH values (6 – 8).

Addition of pyrophosphate ions to the liquid phase also increases setting time at all pH values tested. Smaller concentrations of pyrophosphate ions were necessary to affect setting time, than of tartrate and citrate ions. Addition of 0,01 M pyrophosphate ions prolonged the setting considerably – even twice as much time was needed.

The influence of citrate ions on calcium phosphate cement properties were investigated in detail further, as they could both increase and decrease setting time (depending on initial liquid phase pH)

pH changes in diluted cement pastes

As it was not possible to measure pH changes inside cement paste as it hardened, the cement paste was diluted to suspension. The resulting pH changes in diluted cement pastes were taken as indicative of processes in undiluted cement pastes.

Diluted cement pastes with acidic initial liquid phase pH show the tendency for pH values to increase to slightly basic pH values (pH 7 – 7,5) as

the reactions progressed. Pastes with basic and neutral initial liquid phase pH values retain their pH close to original during reactions, see Fig. 4.

The presence of citrate ions did not affect the pH values of diluted cement pastes significantly.

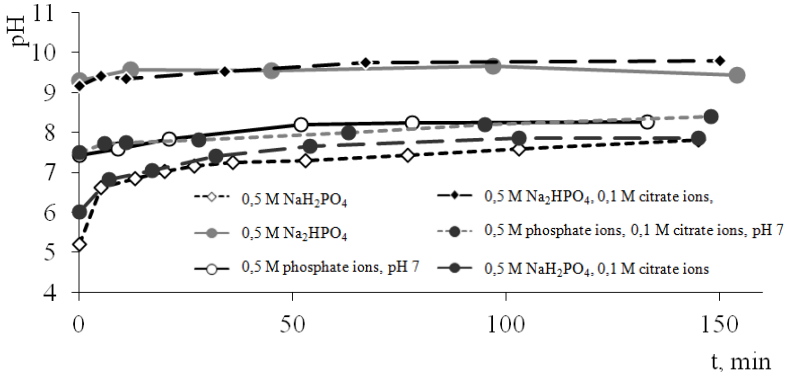

Fig. 4. pH changes in diluted cement pastes at 37°C temperature (the composition of undiluted cement liquid phase is given)

It is shown in literature [8], that DCPD precipitates at pH 2,0 – 6,0, octacalcium phosphate (OCP) precipitates at pH 5,5 – 7,0, calcium deficient hydroxyapatite (CDHAp) at pH 6,5 – 9,5, and stoichiometric hydroxyapatite at pH 9,5 – 12.

Formation of new crystalic phases during cement setting and aging

At first 0.5 h after mixing there are practically no new crystalline phases, only for cements with acidic initial liquid phase pH ($pH \leq 6$) a small amount of DCPD has precipitated, see Fig. 5. It must be taken into account that cements with initial liquid phase pH 8 – 6 are set at this time.

Therefore it can be concluded that cements are set when very small amount (not detectable using XRD) of crystalline phases have precipitated, or amorphous calcium phosphate has precipitated.

After 2 h in cement pastes with acidic initial liquid phase pH ($pH \leq 6$) there is no DCPD, but some OCP precipitation has occurred. For cements with

neutral and basic initial liquid phase pH only starting material can be detected with XRD, see Fig. 6.

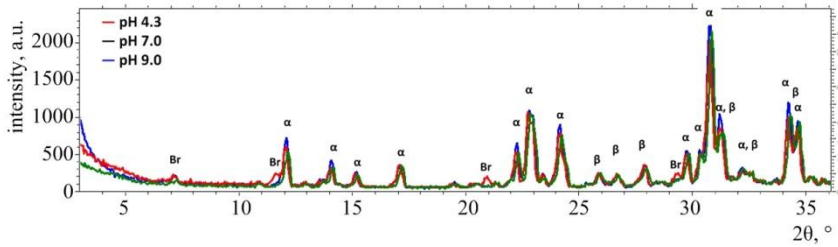

Fig. 5. XRD patterns of cement samples 0,5 h after mixing, without citrate ion additive (α-TCP marked as 'α', β-TCP as 'β', DCPD as 'Br')

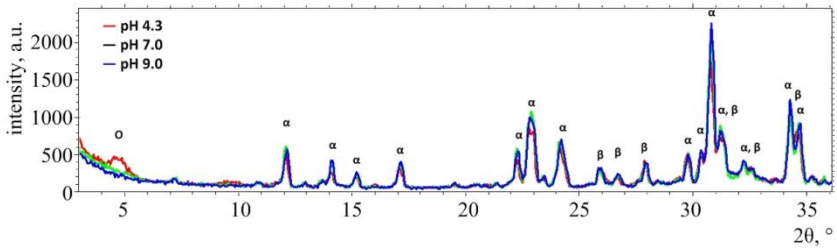

Fig. 6. XRD patterns of cement samples 2 h after mixing, without citrate ion additive (α-TCP marked as 'α', β-TCP as 'β', OCP as 'O')

After 5 h for cements with acidic initial liquid phase pH more OCP has crystallized. For cements with initial liquid phase pH 4,3 – 7,0 at 31 - 34° background area has risen. This can give evidence on the beginning of CDHAp crystallization. For cements with basic initial liquid phase pH (pH ≥ 8) low crystallinity CDHAp has precipitated, see Fig. 7.

After 24 h for cements with acidic initial liquid phase pH (pH ≤ 6) OCP amount is lower, but CDHAp amount is higher. There is more CDHAp crystallized also in other cement samples, see Fig. 8. For cements with more basic initial liquid phase pH larger amount of starting material – α-TCP has reacted, if compared to cements with more acidic initial liquid phase pH, see Fig. 8.

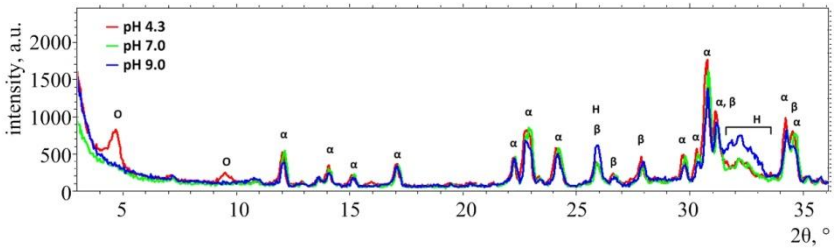

Fig. 7. XRD patterns of cement samples 5 h after mixing, without citrate ion additive (α-TCP marked as ‘α’, β-TCP as ‘β’, OCP as ‘O’, CDHAp as ‘H’)

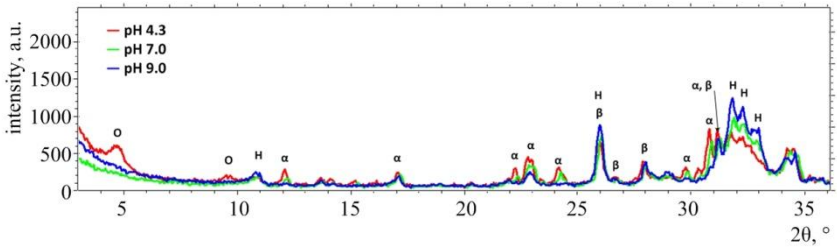

Fig. 8. XRD patterns of cement samples 24 h after mixing, without citrate ion additive (α-TCP marked as ‘α’, β-TCP as ‘β’, CDHAp as ‘H’)

Cements without additives can be alotted into 3 groups: acidic (initial liquid phase pH $\leq$ 6), where firstly DCPD is precipitated, DCP later recystalizes to OCP, which in turn recrystalizes to CDHAp (1), neutral (initial liquid phase pH = 7) where no OCP or DCPD is precipitated, but crystalization of CDHAp starts at aproximately the same time as for acidic cements (2), and basic cements ((initial liquid phase pH $\geq$ 8), where the precipitation of CDHAp and hydrolysis of α-TCP is the most rapid. This allotment was confirmed by XRD analysis. For cements with initial liquid phase pH 4,3 and 6,0 XRD patterns were almost indistinguishable, and the same is true for XRD patterns of cements with initial liquid phase pH 8,0 and 9,0. For cement with initial liquid phase pH 7,0 there is middle amount of CDHAp percipitated and middle amount of α-TCP crystalized, when compared to cements with more basic or acidic initial liquid phase pH.

The XRD analysis also shows, that in presence of citrate ions at acidic initial liquid phase pH there is no DCPD precipitation, but OCP precipitation

and CDHAp precipitation is not affected. If cement initial liquid phase is basic, the presence of citrate ions slows down CDHAp crystalization.

Fig. 9 is a an schematic overview about processes in cements without citric ion additives and crystalline phases percipitating during them.

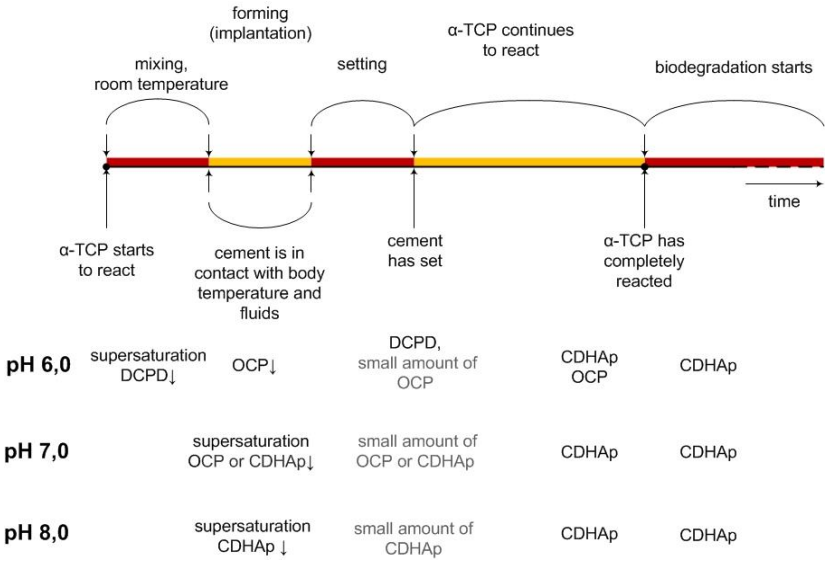

Fig. 9. Processes in cements without citric ion additives and crystalline phases percipitating during them

Initial liquid phase pH and additives affect cement microstucture see Fig. 10 and Fig. 11. Overall crystals percipitated at neutral and basic initial liquid phase pH are smaller, if compared to cements with acidic liquid phase pH. For cements with acidic initial liquid phase pH thin plate-like crystals characteristic of OCP have percipitated.

For 0,5 h old samples in XRD patterns no new phases are detected, if the initial liquid phase pH $\geq$ 7 and only DCPD is detected as a new phase for cements with initial liquid phase pH $\leq$ 6. But in SEM micrographs there are crystals characteristic of OCP and CDHAp seen, see Fig. 10 and Fig. 11. This confirms that new crystalline phases percipitate, but in too small amount to be detected by XRD.

Presence of citrate ions does not significantly change morphology of percipitated crystals, if initial liquid phase pH ≥ 7. If initial liquid phase pH is acidic, in presence of citrate ions morphology of percipitated crystals is different – smaller, see Fig. 11.

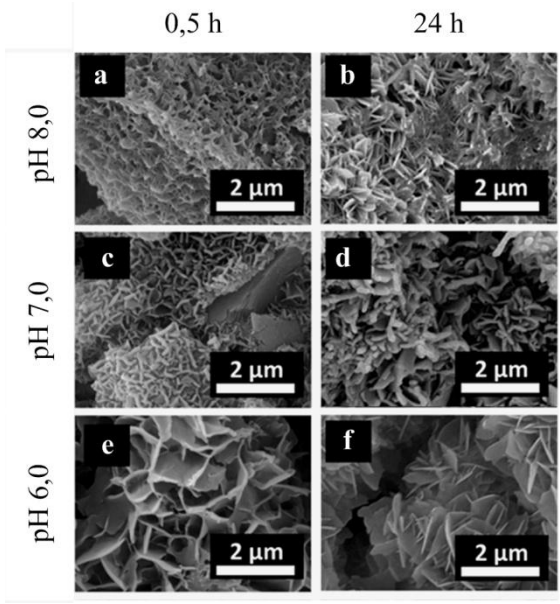

Fig. 10. SEM micrographs for cement samples with different initial liquid phase pH values

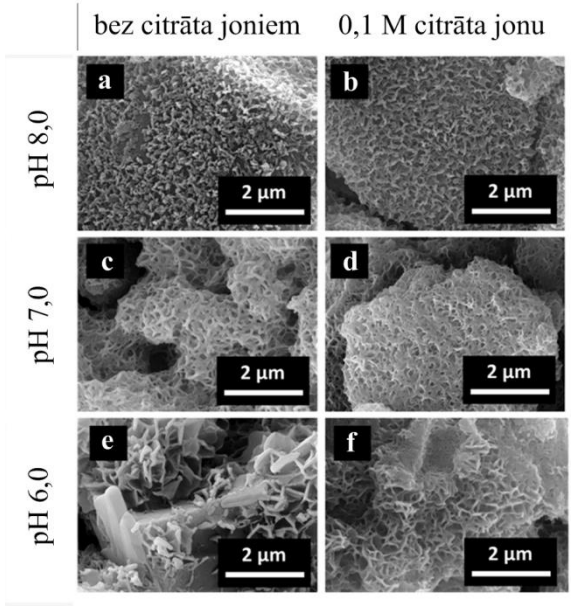

Fig. 11. SEM micrographs for 0,5 h old cement samples with different initial liquid phase pH values and citrate ion additive

Cohesion and injectability of α-tricalcium phosphate based cements

Rheological properties of the calcium phosphate cements include viscosity, cohesion and injectability. The cement paste sets in a few minutes, so the rheological properties of the cement will change quite rapidly.

In general it was observed that cement pastes with a short setting time (less than 40 min at 21°C) exhibited better cohesion than cements with longer setting time. Also other factors (that affect cement setting time) than initial liquid phase pH (phosphate ion concentration in the liquid phase, powder/liquid ratio) can influence cohesion.

In Fig. 12 and Fig. 13 the visual results of cohesion test can be seen – the particles fallen from horizontal cement surface (all with the same area) during incubation in physiological solution at 37°C. There is also the weight of fallen particles given below.

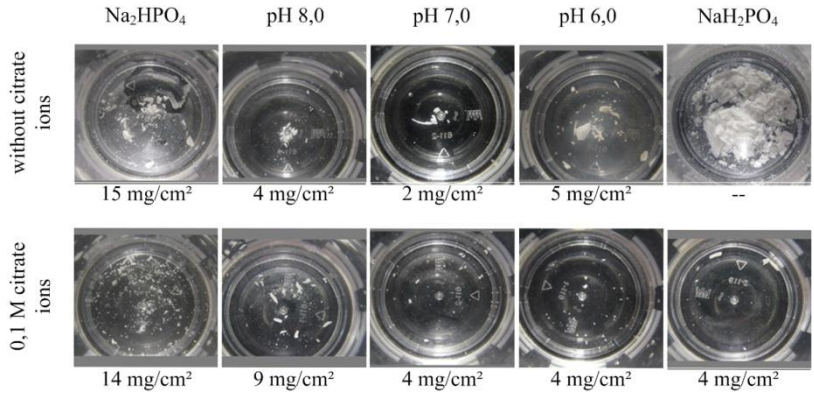

Fig. 12. Visual results of cohesion test – particles falen from surface during incubation in physiological solution at 37°C (powder/liquid ratio – 1,75 g/ml)

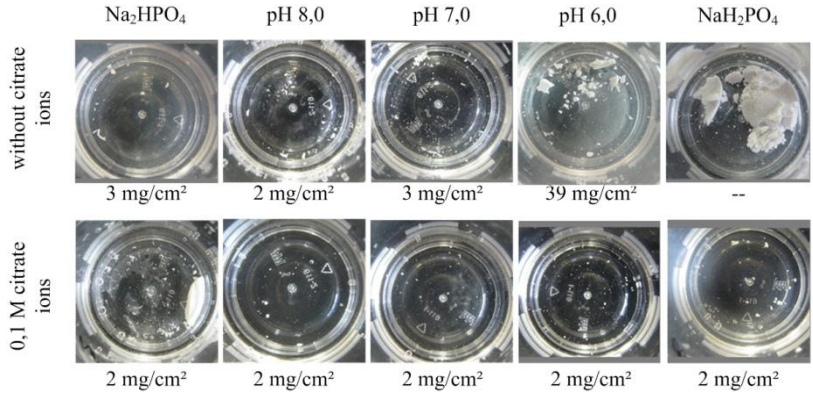

Fig. 13. Visual results of cohesion test – particles falen from surface during incubation in physiological solution at 37°C (powder/liquid ratio – 2,00 g/ml)

For cements with powder/liquid ratio (P/L) 1,75 g/ml, the best cohesion is observed for cement with initial liquid phase pH 7 and without citrate ion additive - there are no powder-like particles and the amount of fallen particles is 2 mg/cm^2.

It can be seen that presence citrate ions improves cohesion for cements with acidic initial liquid phase pH (pH $\leq$ 6) and P/L 1,75 g/ml. It can be explained by shorter setting time in presence of citrate ion additives.

The increase of P/L from 1,75 to 2,00 g/ml improves slightly cohesion for cements with added citrate ions, as well as for cements without added citrate ions and with initial pH ≥ 8, see Fig. 12 and Fig. 13.

Cohesion did not improve for cements with initial liquid phase pH 6, if P/L is increased from 1,75 to 2,00 g/ml. Such cement paste in practice was thick and crumbled.

Liquid phase with comparatively high phosphate ion concentration (0,5 M) and varying initial liquid phase pH, as well as use of citrate ion additive are easily used methods to modify cement setting time. This allows to use cements with low P/L (1,75 g/ml), that are suitable for delivery to implant site with injection.

It was expected that injectability will be higher for cements with longer setting times. This expectation was only partially confirmed. Evaluation of injectability together with cohesion evaluation is shown in Table 1. For cements with P/L 1,75 g/ml injectability was determined for compositions with initial liquid phase pH 6, pH 7 and pH 8. For cements with P/L 2,00 g/ml injectability tests were conducted if cements with the same liquid phase composition had acceptable results with P/L 1,75 g/ml, and also for few other selected compositions.

Contents of Table 1 are colored. Results that would create problems, if cement was applied in practice are colored red, mediocre results yellow, but excellent results – green.

Injectability depends on the same variables as setting time – P/L, initial liquid phase pH and presence of citrate ions.

Some cement pastes with P/L 2,00 g/ml (Nr. 9 and 10) cannot be injected, as phase separation occurs (cement paste extruded from syringe has lower P/L than original pastes), see Table 1.

Cements with P/Š 1,75 g/ml and basic initial liquid phase pH (Nr. 3 and 6). The presence of citrate ions in cement 6 does not affect injectability, when compared to Nr. 6. If P/L is increased to 2,00 g/ml, cement with initial liquid phase pH 8 and without citrate ion additive can still be injectable, but similar cement with citrate ion additive (Nr. 10) – cannot.

Table 1

Cohesion and injectability of cements based on α-tricalcium phosphate[1]

Nr.	Composition of liquid phase	Initial P/L, g/mL	Injected P/L, g/mL	Injected paste, %	Cohesion[2], (mg/cm²)
1	pH 6	1,75	1,62 ± 0,03	82 ± 5	++ 5
2	pH 7	1,75	1,67 ± 0,05	97 ± 2	+++ 2
3	pH 8	1,75	1,73 ± 0,01	98 ± 1	++ 4
4	pH 6, 0,1 M citrate ions	1,75	1,55 ± 0,06	82 ± 4	++ 4
5	pH 7, 0,1 M citrate ions	1,75	1,40 ± 0,05	37 ± 8	++ 4
6	pH 8, 0,1 M citrate ions	1,75	1,66 ± 0,03	98 ± 1	++ 9
7	pH 7	2,00	--	< 10	++ 3
8	pH 8	2,00	1,95 ± 0,02	89 ± 4	++ 2
9	0,5 M NaH_2PO_4, 0,1 M sodium citrate	2,00	1,87 ± 0,03	63 ± 5	++ 2
10	pH 8, 0,1 M citrate ions	2,00	1,86 ± 0,04	67 ± 5	++ 2
11	0,5 M Na_2HPO_4, 0,1 M sodium citrate	2,00	1,95 ± 0,02	91 ± 2	++ 2

[1] – all liquid phase compositions contain 0,5 M phosphate ions

[2] – visual evaluation of cohesion (+++ - very good, ++ - mediocre, + - disintegration)

Cement Nr. 2 has excellent properties, but with added citrate ions (Nr. 5) cement is not injectable. It was also observed, that cement Nr. 2 cannot be injected in larger amounts than tested (2 g of solid phase), as it will then set in syringe, if not injected immediately.

Cements Nr. 2, 3, 6, 8 and 11 have best injectability. Their initial liquid phase pH is basic, except for cement Nr. 2.

Injectability for cements with acidic initial liquid phase pH (Nr. 1, 4, 9) does not reach 90%. This can be explained by precipitation of DCPD during

cement mixing and forming. During mixing of the cement paste it was observed that for cements with acidic initial liquid phase pH cement paste was thicker than for cements with basic initial liquid phase pH.

Compressive strength of cements based on α-tricalcium phosphate

Initial liquid phase pH affects mechanical strength of the cement, see Fig. 14. If initial liquid phase pH is increased, maximum compression strength also increases.

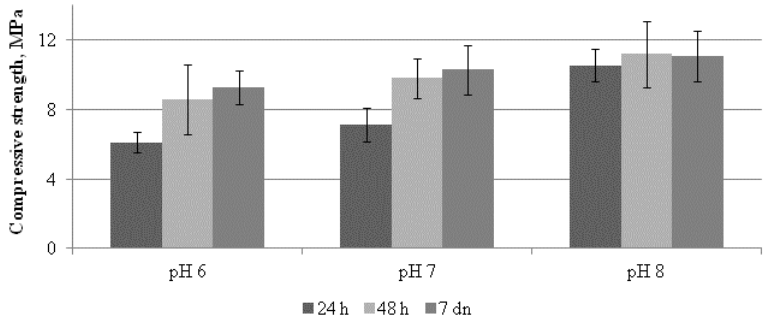

Fig. 14. Impact of initial liquid phase pH and aging time on maximum compression strength of cement samples

Cement with basic initial liquid phase pH (8) achieves highest strength faster than cements with more acidic initial liquid phase pH. After 24 h maximum compression strength of cement with initial liquid phase pH 6 is 6,1 MPa, but with pH 8 – 10,6 MPa. After 7 days for cement with initial liquid phase pH 6, maximum compression strength is 9,3 MPa, but with pH 8 – 11,1 MPa, see Fig. 14.

The reason for these differences between cements with different initial liquid phase pH can be faster α-TCP hydrolysis or the fact that cement pastes with more basic initial liquid phase pH are thinner upon mixing. Thin paste may provide less air bubbles and other defects in the set cement sample.

Cements based on α-tricalcium phosphate as drug delivery device

After implantation of a biomaterial the pain generates in the tissue injury. Therefore the release of local anesthetic drug from the implant is of practical importance. In this chapter the possibility of controlled release of local anesthetic drug – lidocaine (in form of lidocaine hydrochloride monohydrate) – is investigated.

Lidocaine release from calcium phosphate cements with 3 compositions was investigated:

- 'HAp-A': solid phase – α-TCP, liquid phase – 0,5 M Na_2HPO_4, CDHAp precipitates upon setting,
- 'HAp-B': solid phase – α-TCP, liquid phase – 0,5 M NaH_2PO_4, CDHAp precipitates upon setting,
- 'HAp/DCP': solid phase – α-TCP (in excess) and $Ca(H_2PO_4)_2 \cdot H_2O$, liquid phase – 2 M sodium citrate solution, calcium hydrogen phosphate precipitates upon setting, later recrystallizes to CDHAp.

The addition of lidocaine accelerated setting of HAp-A and HAp-B cements (from more than 1 h without lidocaine to 8 min for HAp-A and 29 min for HAp-B).

The release kinetics of lidocaine from cement samples are dependent on cement composition, see Fig. 15. For cements with more acidic nature initial burst release was higher. This can be both because of pH differences (lidocaine is more soluble in acidic pH values) and because of differences between precipitated crystals.

The end of burst release can be correlated with precipitation of new crystals. During first hours more and finer CDHAp crystals are precipitated in HAp-A and HAp-B cements than calcium hydrogen phosphate crystals in HAp/DCP cements.

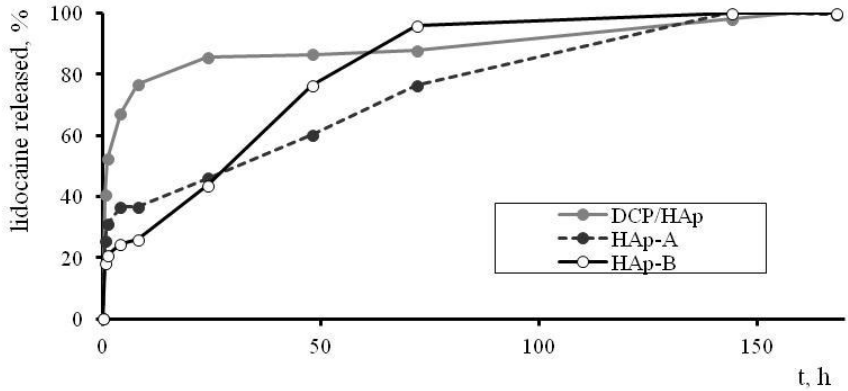

Fig 15. Release of lidocaine from cement samples containing initially 30 mg lidocaine per 0,7 g of cement solid phase.

In vitro and in vivo tests

In *in vitro* tests it was found that cells attached to all samples. Using MTT test it was ascertained how many live cells were attached to cement surface (compared to control material) after 72 h of incubation.

Although cells attached at least in small amounts to all cement samples, there were significantly more (even 50-100 times) cells on cement sample surface with initial liquid phase pH 7. On cement samples with basic or acidic initial liquid phase pH significantly fewer cells were attached. It must be noted that cement samples were aged two weeks in phosphate buffered saline, therefore no adverse change of pH in cell growth media occurred.

In *in vivo* tests it was ascertained that 3 months after implantation in rabbit bone tissue all tested cement samples were almost completely biodegraded and replaced with healthy bone tissue. There were no significant differences between cement compositions.

CONCLUSIONS

1. If pH of the cement liquid phase is changed, it affects cement setting time:
    a) if no citrate ion additive is used, the fastest setting (as fast as 5 – 7 minutes) occurs in the initial pH of cement liquid phase is 7,
    b) if no citrate ion additive is used, setting time can be increased by 5-20 min if initial pH of cement liquid phase is increased or decreased to 6 and 8, respectively,
    c) if citrate ion additive is used, the fastest setting time is, if initial liquid phase pH of the cement is 6 and setting time can be prolonged, if initial liquid phase pH is more basic.
2. Cohesion of the cement paste is linked to setting time. The best cohesion is observed for samples with fastest setting times and without citrate ion additive.
3. Initial cement liquid phase pH has an effect on phase composition, morphology of the set cement and on processes during cement aging. Fastest (complete during 24 h) hydrolysis of α-tricalcium phosphate occurs if initial liquid phase pH is 8 – 9.
4. Burst release of lidocaine is smaller from cement that precipitates calcium deficient hydroxyapatite upon setting, than from cement that precipitates calcium hydrogen phosphate dihydrate (brushite) upon setting.
5. Cements based on α-tricalcium phosphate with initial liquid phase pH 7 and without citrate ion additive have the biocompatibility, if tested using MG63-GFP cell line.
6. For implantation as a cement paste, the most suitable is cement composition with initial liquid phase pH 7 and without citrate ion additive.
7. For minimally invasive implantation (injection) of cement, the most suitable is cement with initial liquid phase pH 8 (with or without citrate ion additive).

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