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THE INFLUENCE OF POLYMER ADDITIVES ON THE STABILITY, BIOMECHANICAL AND MORPHOLOGICAL PROPERTIES OF RESORBABLE BONE CEMENTS

VLIV POLYMERNÍCH ADITIV NA STABILITU, BIOMECHANICKÉ A MORFOLOGICKÉ VLASTNOSTI RESORBOVATELNÝCH KOSTNÍCH CEMENTŮ

MASTER'S THESIS
DIPLOMOVÁ PRÁCE

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Abstract

Calcium phophate cements are widely used in health care like a bone repair material. In present times their succes in wider aplication is limited by mechanical properties of these cements. Especialy the compresive strength and the adhesion to human bone is not so satisfactory and enforceable to fully replace recent materials used in medicine.The aim of this study was improve the mechanical properties of bone cements by using properly choosed additives.

Keywords

Calcium phosphate cement, compressive strength, polymeric aditives

Abstrakt

Vápenno-fosfátove cementy su široko využívané v zdravotnictve ako materiál na náhradu kosti.
V súčasnosti ich úspech v širš uplatnení je limitovaný mechanickými vlastnosťami týchto cementov. Špeciálne sila v tlaku a priľnavosť ku ľudskej kosti nie je uspokojivá a dostačujúca na to aby plne nahradila súčasné využívane materiály. Cieľom študie bolo zlepšiť mechanické vlastnosti kostných cementov tým že sa dosledne vhodne zvolia aditíva.

Kľúčové slová

Vápenno-fosfátové cementy, pevnosť v tlaku, polymérne aditíva

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POĎAKOVANIE

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1 INTRODUCTION

Calcium phosphate biocements based on calcium phosphate chemistry are well-established biomaterials for the repair of non-load bearing bone defects due to the brittle nature and low flexural strength of such cements. This kind of biomaterial is promising tool in medicine to improve patient well-being and recovery time after injury. However some of their properties such as britllenes and low fracture toughness can limit the use of CPC to non-load-bearing aplications. Common approaches to reduce brittleness of CPC and to improve their mechanical performance for load-bearing applications cover the modification of the cement liquid with polymeric additives such as collagen the addition of fibres to the cement matrix or the use of dual-setting cements in which a dissolved monomer is simultaneously cross-linked during cement setting. Aim of this study was improve stability, biomechanical and morphological properties of these materials by using polymeric aditives.

2 TEORETICAL PART

Calcium phosphate cement (CPC) based systems are self-setting bioactive biomaterials. To expand the clinical applicability of calcium phosphate cements (CPCs) to load-bearing anatomical sites, the mechanical properties of CPCs need to be improved. Specifically, organic additives need to be developed that can overcome the disintegration and brittleness of CPCs. The risks associated with the use of CPCs as bone substitutes are related to the disintegration and brittleness of CPCs. For example, premature disintegration can result in inflammatory responses [4]. In addition, these disintegrated cement particles may leak into the tissue surrounding the defect area, causing side effects such as nerve pain, venous and pulmonary embolism .Concerning the brittleness of CPCs, it was shown in [8] that the flexural strength of CPC is low compared to bone, thereby limiting the applicability of CPCs to non-load-bearing anatomical sites

2.1 Bone

The human skeleton is designed to protect vital organs and serve as a framework for muscles and locomotion. In addition, bone acts as a reservoir for minerals, such as calcium and phosphate, and regulates the concentrations of the mineral ions in the body. The cavities of the larger bones contain bone marrow, which is the major source of multipotent cells and the primary site for the production of blood cellular components.

2.2 Bone structure

Bone has anexceptional toughness and strength, which enables it to with-stand high loads without breaking. The mechanical properties of bone are based on its unique material composition and the three dimensional organisation of the bone structure. Bone is composed of roughly 60% inorganic calcium phosphate minerals, which primarly includes calcium deficient hydroxyapatite; 30% organic material, which is mainly collagen and 10% water. While the collagen provides toughness to the bone, the minerals contribute to its strength [12, 13]. Based on the macroscopic structure, one can distinguish two types of bone tissue. The first type is highly dense cortical bone, which has 10% porosity and relatively few cells. Cortical bone is composed of structural units of osteons, which can be described as cylinders with several bone layers arranged around a Haversian canal; this canal is a cavity with blood vessels and nerves (Fig 1). The osteons are aligned in the longitudinal direction of the bone and can thus provide higher strength in that direction. The second type of bone is trabecular bone, which has a porosity of 50–95% [13]. It is more metabolically active than cortical bone due to its larger surface area and higher number of cells.

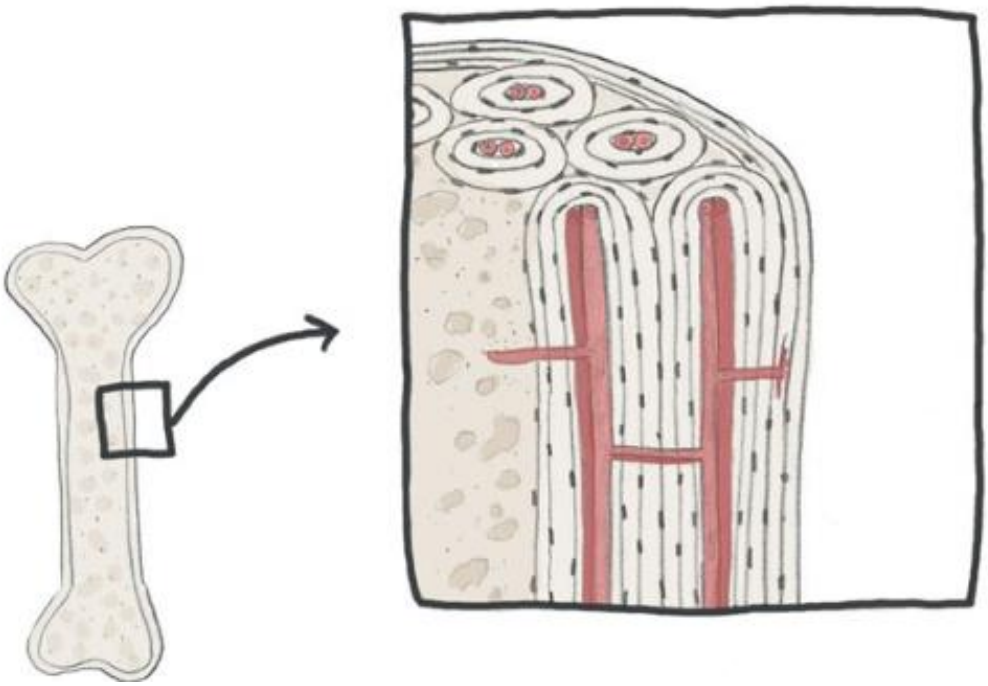

Fig 1 : *The illustration shows the structures of cortical bone, which is composed of cylindrical-shaped osteons arranged around Haversian canals containing blood vessels*

2.3 Bone remodeling

Bone is a dynamic tissue that is actively renewed throughout a lifespan. The strength and integrity of bone depends highly on the repair of microcracks that arise upon load. Bone cells are the machinery responsiblefor the repair. The maintenance of bone is known asremodeling,and it implies the removal of old bone matrix by osteoclasts and the production of new bone matrix by osteoblasts (Fig 2). It is a highly regulated process with crosstalk between the different types of bone cells. This crosstalk is critical to achieve a balance between bone resorption and bone formation. If the remodeling balance is disrupted, pathological disorders can occur, such as osteopenia or osteoporosis involving a net loss of bone. At the other extreme, osteopetrosis is a disorder that involves a net excess of bone [13, 14]. Three highly specialised cell types are associated with bone tissue.

2.3.1 Osteoblast

Osteoblasts are responsible for the production of bone matrix. They are derived from multipotent mesenchymal stem cells (MSC), which can also differentiate to other cells, such as adipocytes (fat tissue cells) and myoblasts (muscle tissue cells) [16, 17].

When osteoblasts have ended their matrix production activity, they have one of three possible outcomes: apoptosis (cell death), becoming lining cells (resting state) or differentiating into osteocytes [17, 18]

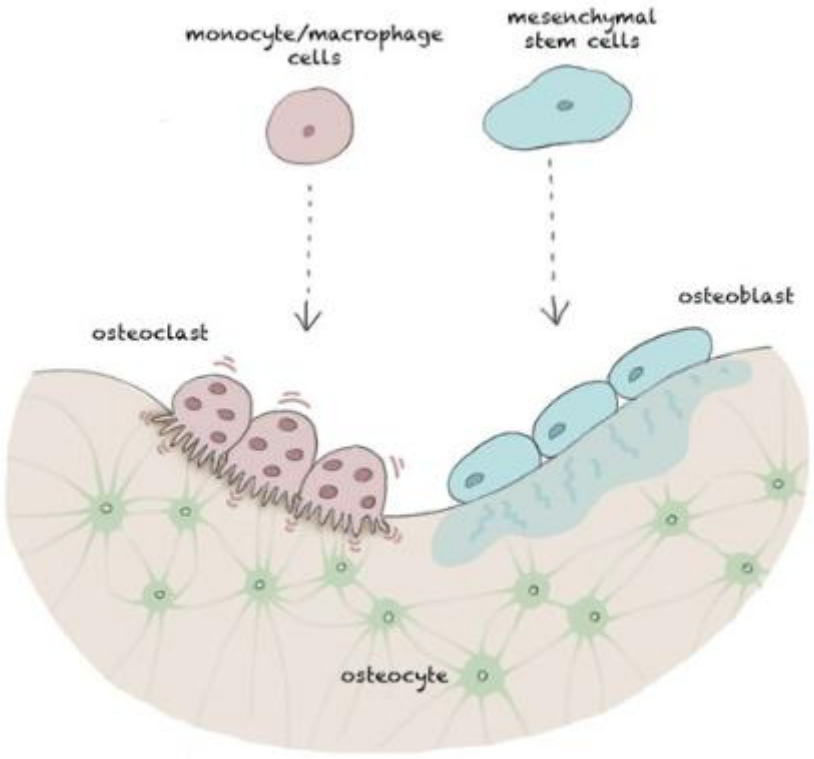

Fig 2: *Osteoclasts are derived from monocyte/macrophage cell lineage and re-sorb bone matrix, while osteoblasts are derived from MSCs and produce new bone matrix. The osteocytes are embedded within the bone matrix and are responsible for signal transduction*

2.3.2 Osteoclast

Osteoclasts degrade bone matrix in a process known as bone resorption. They are derived from hematopoietic cells of a monocyte/macrophage lin-age. Osteoclasts are large multinucleated cells formed by the fusion of several osteoclast precursor cells [19]

2.3.3 Osteocyte

Osteocytes are mainly responsible for signal transduction. They are the most abundant cell type in bone, and they have a lifespan of up to several decades, in contrast with other bone cells that only live for a few weeks [18]. Osteocytes are evenly distributed within the bone matrix and communicate with each other by dendritic extensions that run along smallliquid-filled channels known as canaliculi [14]. The dendritic extensions are developed during the differentiation from osteoblast to osteocyte, andeach cell can have up to 50 extensions[17, 18]. Through the complex network of canaliculi, the osteocytes have the ability to sense mechanical load because it changes the fluid flow inside the channels. In response to these signals the osteocytes secrete factors to regulate osteoblast and osteoclast activities [14]

2.4 Calcium phosphate cements

2.4.1 History of CPC‘s

The first scientific study on the use of calcium phoshate for bone defects may be the study of Albee and Morrison in 1920 when triple-calcium phosphate was used as stimulus for bone growth. The results indicated that bone fractures, with bone loss showed a more rapid growth and union when triple calcium phosphate was injected into gap between the bone ends than did the controls without its use. [1]. Ceramic hydroxyapatite (HA) repair was first reported in early 1950s by Ray and Ward. [1] Granular calcium phosphate materials have well established biocompability and osteoconductivity. Their clinical use as been partially replaced by calcium phosphate cement materials because of the advantages associated with self-hardening properties of the cements. However granular materials remain highly useful when used alone or as osteoconductive fillers in polymeric tissue scaffolds or calcium phosphate cements implants. [1] In early 1980s , studies on the reaction TTCP (tetracalcium phosphate) + DCPD (dicalcium phosphate dihydrate) = HA (hydroxyapatite), were conducted with the aim of developing slurries for remineralizing carious lesions. It was observed that some of the TTCP + DCPD aqueous pastes became a hardened mass when left in test tubes for a few hours. These scientists discovered a new type of self-hardening cements that consisted of only calcium phosphates and formed HA as the product. [1]. In subsequent years results from a series of animal studies demonstrated that because CPC formed biocompatible, precipitaded nano-crystalline HA,as the product, implanted CPCwas gradually replaced by new bone without a loss in volume. This CPC composition received approvalby the US FDA in 1996, becomig the first comercially avaible CPC for use in humans. [1] The discovery of first self-hardening calcium phosphate cements (CPCs) in 1982-1983 opened up a new era of bone substituting materials.[2]

2.4.2 Chemistry of CPC setting

Calcium phosphate cements (CPCs) are self-setting bioactive materials with unique properties for bone regeneration applications. Calcium phosphate cements (CPCs) are used for this purpose as injectable materials to fill bone voids and to improve hardware fixation in fracture surgery. [3] CPCs form a biomaterial that is chemically similar to the mineral content of human bone possessing biocompatible and osteoconductive properties. Calcium phosphate cements (CPCs) should ideally have mechanical properties similar to those of the bone tissue the material is used to replace or repair. Usually, the compressive strength of the CPCs is reported and, more rarely, the elastic modulus. [4] However, the strength and stiffness of the osseous tissue can vary substantially, not only depending on anatomical site and within different regions of the same bony site, but also among individuals, with age, sex, activity level and, due to different pathologies.[4]

CPCs are produced by mixing a powder and liquid component to form a paste, which sets to form a solid material. The powder components typically consist of different CaP and a large range of powders have been used. CPCs normally set via a dissolution–precipitation process (Fig:3). [23]. The initial materials dissolve in solution, after which an entanglement of crystals precipitates, thereby providing mechanical stability and the mechanism of hardening .[5] Cement setting reaction is perhaps the most

important properties of CPC because it not only directly controls cement hardening time and other setting properties but also determines the nature of cement products and therfore most of the physical and biological properties of the hardened cement.[1].

Due to the high level of interest and research into CPC, many different formulations of CPC have been developed. They can be divided into two principal groups: (1) apatite (hydroxyapatite, HA, and calcium-deficient HA, CDHA) and (2) brushite cements (dicalcium phosphate dihydrate, DCPD)(Fig. 4) [21]. Both apatite and brushite CPC are produced by mixing a powder component consisting of one or more calcium orthophosphates with an aqueous solution. The mixing of these two phases induces the dissolution of the initial calcium orthophosphates. This is followed by precipitation into crystals of HA, CDHA or DCPD. During precipitation the newly formed crystals grow, and it is the entanglement of these new crystals, providing mechanical rigidity, that causes the cement to physically harden or set [20]. The mechanical strength of a cement matrix is a direct result of this crystal entanglement and several factors determine the final strength of the matrix, such as degree of conversion, setting product or porosity[41].

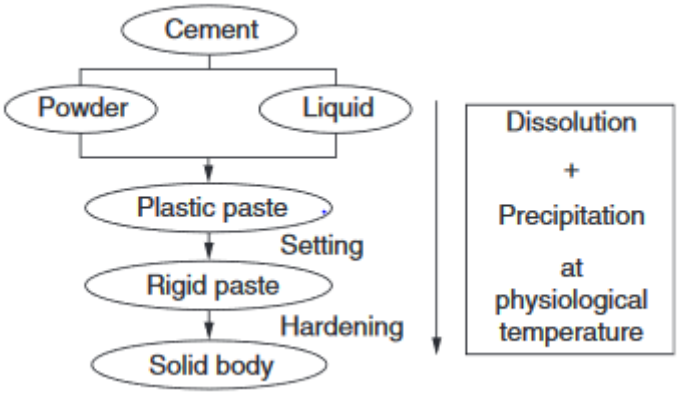

Fig:3 : *The cement sets as a result of a dissolution and precipitation process* [23].,

CPCs may be divided into the following categories in terms of cement setting reactions:

1. Hydroxyapatite (HA) can be formed via an acid-base reaction of tetra-calcium phosphate, TTCP (basic), and dicalcium phosphate anhydrous, DCPA (slightly acidic), Eq. (1).:

$$Ca_4(PO_4)_2O + CaHPO_4 \rightarrow Ca_5(PO_4)_3OH \quad (1)$$

   All reactions between calcium phosphate compounds that occur in an aqueous enviroment can be characterized as dissolution/re-precipitation reactions

2. Hardening of the cement is a result of reaction between calcium and carboxylic acid
   Calcium deficient HA (CDHA) can be obtained via the hydrolysis of a metastable CaP e.g. α-tricalcium phosphate (α-TCP), Eq. (2).

$$3\alpha - Ca_3(PO_4)_2 + H_2O \rightarrow Ca_9(HPO_4)(PO_4)_5OH \quad (2)$$

A number of carboxylic acids readily form calcium complexes as well as relatively insoluble and often amorphous Ca- carboxylate compounds. These acids include glycolic, citric, tartaric malonic, malic, succinic and maleic acids.

3. Cement hardening is a result of reaction between calcium phosphate and an aqueous polymer solution

Brushite (slightly acidic) can be obtained for instance by a reaction between β-TCP (almost neutral) and monocalcium phosphate monohydrate, MCPM (acidic), Eq. (3) [20].

$$\beta - Ca_3(PO_4)_2 + Ca(H_2PO_4)_2 \cdot H_2O + 7H_2O \rightarrow 4Ca(HPO_4) \cdot 2H_2O \qquad (3)$$

The hardening is a result of acid-base reaction between the carboxylic groups of the polymer and the alkaline calcium phosphate.

In theory, all CaP compounds could be used to form CPC, however there are only three main cement compositions produces. (1) CDHA and (2) DCPD. (3) DCPA (dicalcium phosphate anhydrous) Therefore , it has transpired that there are three major groups of CPCs:

1. **Apatite cements**
2. **Brushite cements**
3. **Monetite cements**

Apatite CPCs are cements that form CDHA (calcium deficient hydroxyapatite) as the end product. The majority of research has concentrated on apatitic cements due to the similarity of CDHA to the mineral content of bone.

Brushite CPCs are cements that produce DCPD (dicalcium phosphate dehydrate) as the end product via an acid-base raction that is slightly acidic material [5]

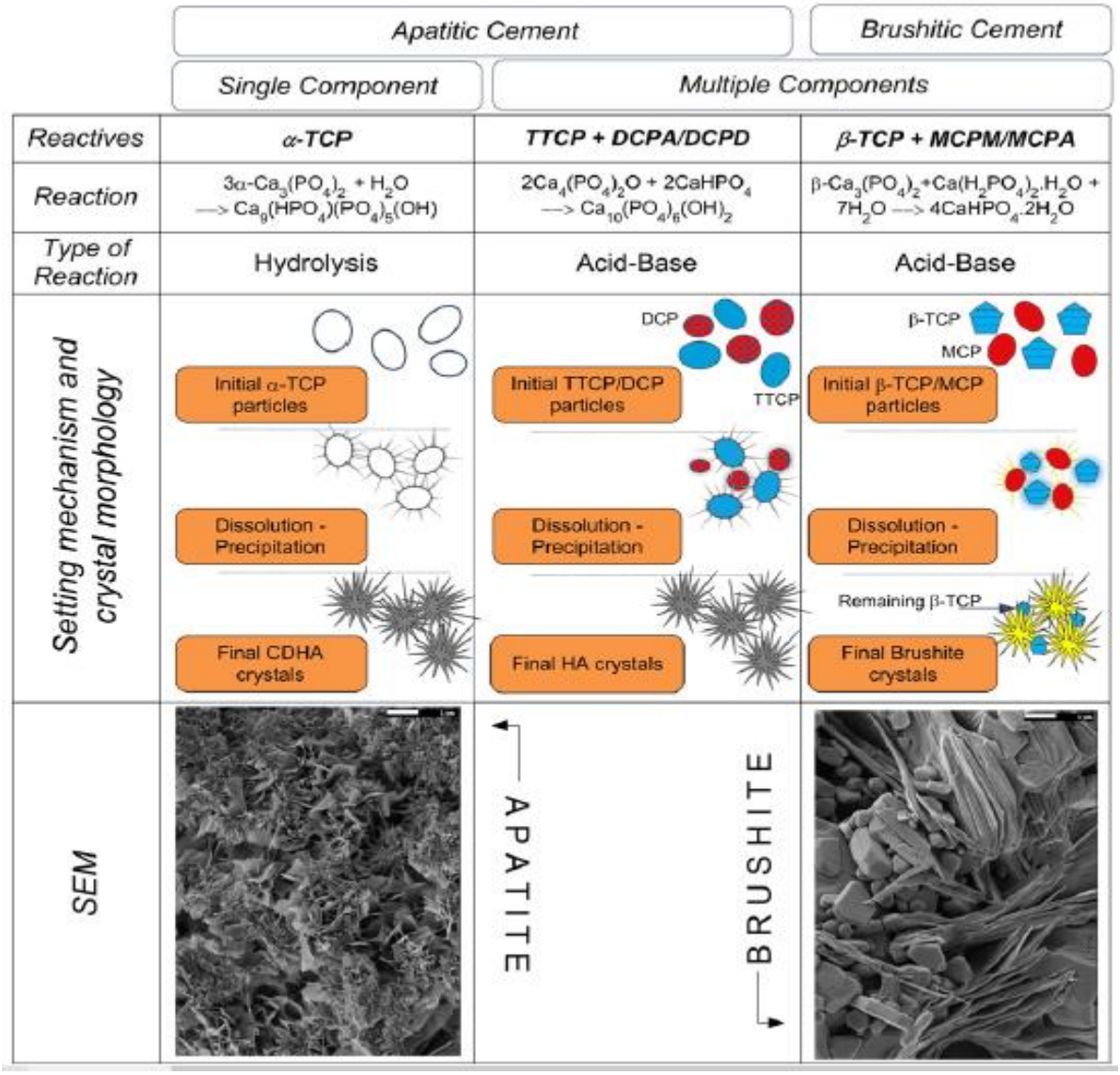

Fig. 4: *Classification of calcium phosphate cements, with examples of the most common formulations. From top to bottom the cements are classified by the type of end-product(apatite or brushite), number of components in the solid phase (single or multiple), type of setting reaction (hydrolysis or acid–base reaction), setting mechanism and microstructure evolution during setting. Scanning electron micrographs of set apatite and brushite cements obtained by the hydrolysis of α tricalcium phosphate (α-TCP) and by reaction of β-TCP with MCPM (monocalcium phosphate monohydrate) respectively, are also shown.* [22]

2.4.3 Kinetics of CPC setting

At present, there are numerous combinations of calcium- and phosphate-containing compounds in CPCs. [25,26].

Understanding the mechanisms controlling the setting process of CPCs will help to gain a comprehensive knowledge about their setting kinetics and then to better control their microstructure, which determines their applications for different purposes. Currently, many CPC substitutes are based

on the hydrolysis of an α-tricalcium phosphate (α-TCP) powder, which is used in most commercial cement formulations.

Many studies have focused on the effects of particle size [28], crystallinity [29], temperature [30] and various constituents [31]. Many methods, such as X-ray diffraction (XRD) [32], isothermal calorimetry [28], strength measurement [32], solid-state nuclear magnetic resonance imaging (NMR) [33], impedance spectroscopy [34] and attenuated total reflectance–Fourier transform infrared spectroscopy [35] have been used to study the evolution of setting reaction with time.

Fernandez et al. [36] estimated the extent of conversion of α-TCP to CDHA by using the height of several selected peaks obtained by XRD, assuming a quasi-constant ratio between peak height and peak area. Ginebra et al. [27] calculated the relative amounts of different phases existing in the specimen by using an external standard method. Both groups of authors found that the extent of conversion of α-TCP to CDHA could be exponentially fitted as a function of hardening time.

Ginebra et al. [32] investigated the effect of different particle sizes of α-TCP on the kinetics of the setting reaction by combining data from XRD and strength measurements. The results showed that fine particles have a much faster rate of hydrolysis than coarse ones. This could easily be explained by considering the fact that a higher specific area accelerates the process of dissolution.

To sum up, there are many factors, such as particle size, crystallinity, temperature, composition and even physical modification of the reactant surface [37], influencing the kinetics of α-TCP setting. Due to chemical similarity, it is expected that these factors should also play an important role in the kinetics of the setting reactions of other cement systems [38] Furthermore, besides the methods mentioned above, other methods (e.g. measurement of rheological properties, pH and calcium (phosphate ion concentration) might be desirable to characterize the kinetics of CPC setting [38].

2.4.4 Properties

Besides having excellent biological behavior, being injectable and self-setting in vivo at body temperature are the two main advantages of CPCs as bone substitutes. However, without any improvement, CPCs normally have a relatively long setting time, poor injectability and poor cohesion[39]

When designing CPC for orthopaedic applications the properties of the unset and set cement require careful consideration to ensure clinical success. The hardened cement must be biocompatible and have sufficient mechanical integrity to stabilise the fracture or implant site. Ideally the hardened CPC should have a suitable composition and adequate porosity to be bioresorbed and replaced by host tissue. The cement prior to setting has to be easily prepared and handled during the surgical procedure.[24]

Some of the main limitations of CPCs are their brittleness and their poor resistance to tensile forces, which limits their use in clinical applications. Moreover, as for most porous ceramic materials tensile tests are difficult to set-up for CPCs.[4] The porosity of CPCs is an important factor since it influences the degradation rate of the cements and also has a direct negative effect on the mechanical properties [3] Several studies have investigated the influence of porosity on the compressive strength and diametral tensile strength of CPCs [3,7].

Concerning the brittleness of CPCs, the flexural strength of CPC is low compared to bone. To broaden the application of CPC to load-bearing applications such as spinalfusion a toughened CPC with an increased fracture toughness needs to be developed. [6] . The most common approach to reduce the brittleness of CPC involves the modification of the cement liquid with polymeric additives.

The most common mechanical assessment of CPCs is quasi-static compression testing, which is not dependent on fixation and specimens are easy to prepare. [4] It has been demonstrated that the compressive strength varies greatly between different types of CPCs due to differences in chemical composition, particle size of starting powders, additives, liquid to powder (L/P) ratio, sample preparation, resulting porosity, storage and testing conditions (*e.g.*, testing dry or wet cements) [4]

2.4.5 Modiffications of CPC bone cement

A reduction of the brittleness of CPC and an increase of strength can be achieved by using polymeric compounds which can be cross-linked by binding calcium ions due to a high density of either carboxylic acid or organic phosphate moieties in the polymer chain. [41]

Some studies has shown that different modifications of CPC can improve some of their main qualities such a mechanical strength, flexibility adhesion, antibacterial properties and many more.

To improve mechanical properties it was prepared numerous polymeric aditives. For example: In study [8] were synthesized injectable citrate-based musel inspired bioadhesives, in study [9] were incorporated chitosan microspheres into cement solid phase and according to study by [10] was added cross-linked chitosan into CPC to improve mechanical properties.

3 GOALS OF THE WORK

The aim of this study was to modificate the biomechanical properties of ceramic bone cements with polymeric additives in order to improve their mechanical properties. Main goals of the work were:

1. Literature searching on biomechancs and structure of bone phosphate cements
2. Methodology of sample preparation, including modifications by additives
3. Monitoring of setting and subsequent degradation (physiological conditions at 37 °C)
4. Biomechanical testing of samples + morphology
5. Evaluation, discussion and conclusion

4 EXPERIMENTAL PART

4.1 Chemicals

4 types of cements were prepared in this study. The following chemicals were used to create these cements.

Cements without polymeric additives:

Refference cement (Ref) : (triblock PLGA–PEG–PLGA copolymer, α-TCP, MCP)

Activated cement (Activ) : (triblock PLGA–PEG–PLGA copolymer, pre- milled α-TCP, MCP)

and a *cements with additives:*

Cement with Dopamine (Ref / DOPA) : (triblock PLGA–PEG–PLGA copolymer, TRIS , DOPA , MCP, α-TCP ,)

Cement with Dopamine and $NaIO_3$ (Ref / DOPA+I) : (triblock PLGA–PEG–PLGA copolymer, TRIS , DOPA , MCP, α-TCP and $NaIO_3$

4.2 Methods

4.2.1 XRD

After the mechanical tests, solid samples were grinded to powder and X-ray diffraction was applied to determine the crystal phases of the cement composites. The kinetics of the transformation from the original α-TCP to calcium-deficient hydroxyapatite (CDHA) of the CPC was studied by means of Wide-Angle X-ray Scattering Analysis (WAXS, Rigaku Miniflex 6000, Tokyo, Japan). Following SEM observation, the remaining set cement samples were milled and analysed. The diffraction patterns of the analysed samples were collected and evaluated employing PDXL software.

4.2.2 SEM

The fracture surfaces after the bending tests were collected for morphological analysis using scanning electronmicroscopy. The morphology of the cements was observed by a scanning electron microscope (SEM, Tescan Mira3, Brno, Czech Republic). All the observations were made in the secondary electron emission mode with a 10 kV acceleration voltage, beam intensity of 10 and a working distance of 15 mm.

4.2.3 Mechanical Testing

Compression testing was performed on the cement specimens using mechanical testing equipment (Zwick Roell 022, Ulm, Germany) with a load-cell of 1 kN, a crosshead speed of 1 mm min^{-1} and a pre-load of 10 g.

4.2.4 Rheological Analysis

The rheological properties and tests were conducted in steady mode using a controlled-stress rheometer (AR-G2, TA Instruments)

4.2.5 Statistycal analysis

The mean values were calculated for the number of readings (n) from each experiment; the error bars refer to the standard deviation (SD).

4.3 Preparation of samples

The experimental cement was prepared by mixing calcium phosphate based powders with a liquid phase to create a paste which hardens into a cement for a short period of time. The starting powder contained monocalcium phosphate monohydrate (MCP, Sigma-Aldrich) and α-tricalcium phophate (α -TCP, Premier Biomaterials) in a 2:98 weight ratio. The liquid phase was an aqueous solution of polymer (triblock copolymer , Ceitec Brno) of 20% by weight. As solvent was used deionized water. A liquid-to-powder ratio of 0,5 mL/g was used in this process. The powders were pre-dried at 110 °C for 24 hours. To prepare the cement paste, the starting powder and the liquid phase were put in a beaker and mixed for 60 seconds.

Modified cements are prepared as aforementioned mechanism with some different substances in liquid and solid phase :

Cement modified with Dopamine (Ref/Dopa):

Liquid phase : ABA- triblock copolymer (20 w% polymer solution)

Dopamin (40 mg/ml)

TRIS (10mM/ml)

Solid phase MCP (2 w%)

α-TCP (98 w%)

Cement modified with Dopamine and Iodine (Ref/Dopa+I):

Liquid phase : ABA- triblock copolymer (20 w% polymer solution)

Dopamin (40 mg/ml)

	TRIS (10mM/ml)
Solid phase	MCP (2 w%)
	α-TCP (98 w%)
	$NaIO_3$ (8 w% this is replaced from Dopamine amount in liquid phase)

4.2.1 Optimization process

The cement pastes were moulded in rubber moulds with approximately 6 mm in diameter and 12 mm in height for commpresive strenght (CS) tests. The cements was set for 24 hours in an absolutely ethanol to remove remaining water content. After that the obtained hardened cements samples were polished, in order to make the two ends flat and parallel and achieve a height of 12 mm according to the standard for acrylic bone cements.[11] Dimensions of samples for CS tests were measured after drying using a caliper. The specific dimensions are presented in

Tab 1. The sample sizes were inserted into the measuring device program. Commpresive strenght was measured using cylindric samples.(Fig:6) The testing machine measured the CS at a cross-head speed of 1 mm/min until failure. The recorded compressive strength was calculated by using PDXL2 software of testing machine.

The aim of the optimization process was to find out whether the form in which are the bone cements prepared affects the compressive strength due to ability to get moisture to samples through different level of barrier during the hardening process.

The study of CS has shown that all samples from 3 different types of moulds has nearly the same value of CS.

In total 6 CPC specimens for every type of CPC were prepared at a fixed liquid to powder (L/P) ratio of 0.5 for all types . After mixing, the pastes were injected into a PTFE mold(cylinder shaped, diameter=6 mm, height=12 mm) by 2 ml plastic syringe, to obtain cylindrical-shaped samples. Then the specimens were put to plastic container with destilled water and put to incubator for appropriate time at 37˚C. The specimens were removed from the moulds after specific period of time and prepared for subsequent tests.

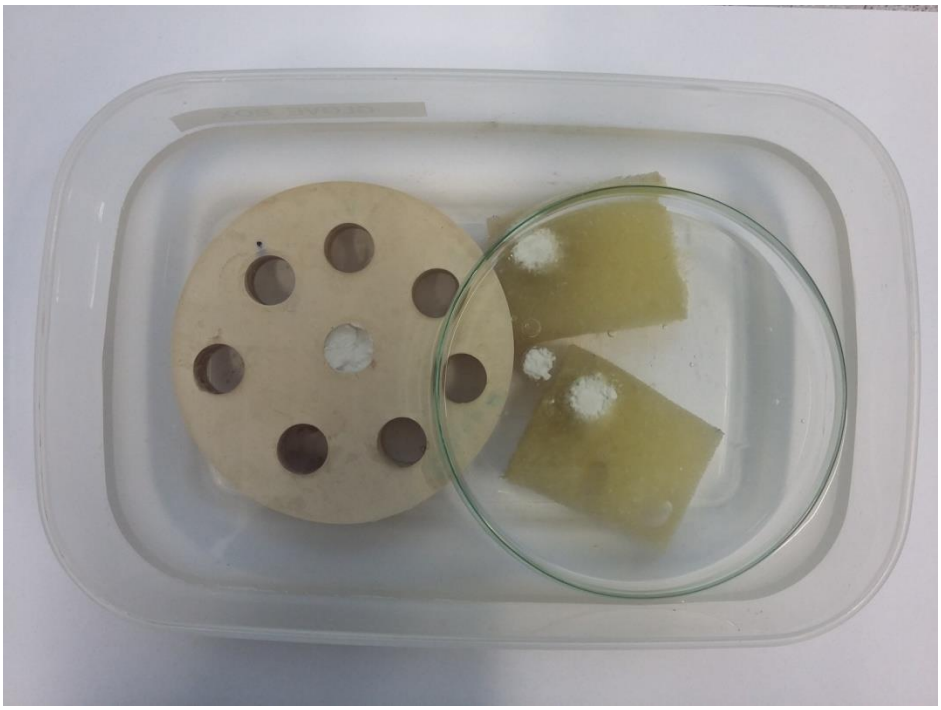

Fig 5: 3 *Types of moulds (rubber, sponge, rubber tube) used as forms filled with cement paste*

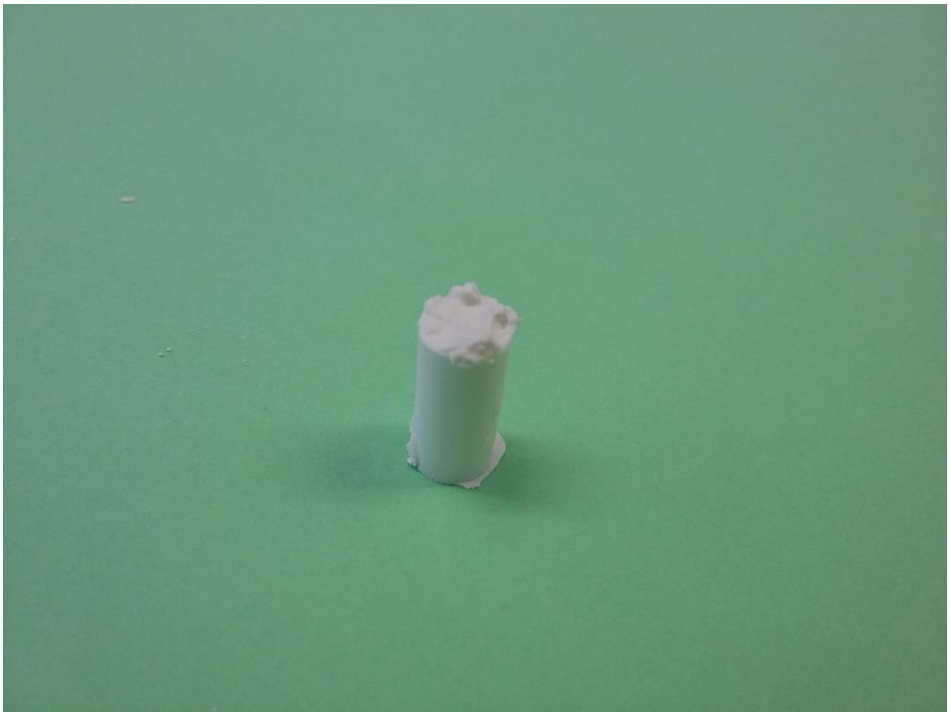

Fig:6 *Cylindric shaped sample of hardened cement*

Tab 1: The dimensions of prepared cement samples

Sample	**Rubber tube**	**Sponge 1**	**Sponge 2**	**Rubber**
Height [mm]	11,57	12,77	11,71	10,55
Diameter [mm]	6,32	5,71	5,88	5,86

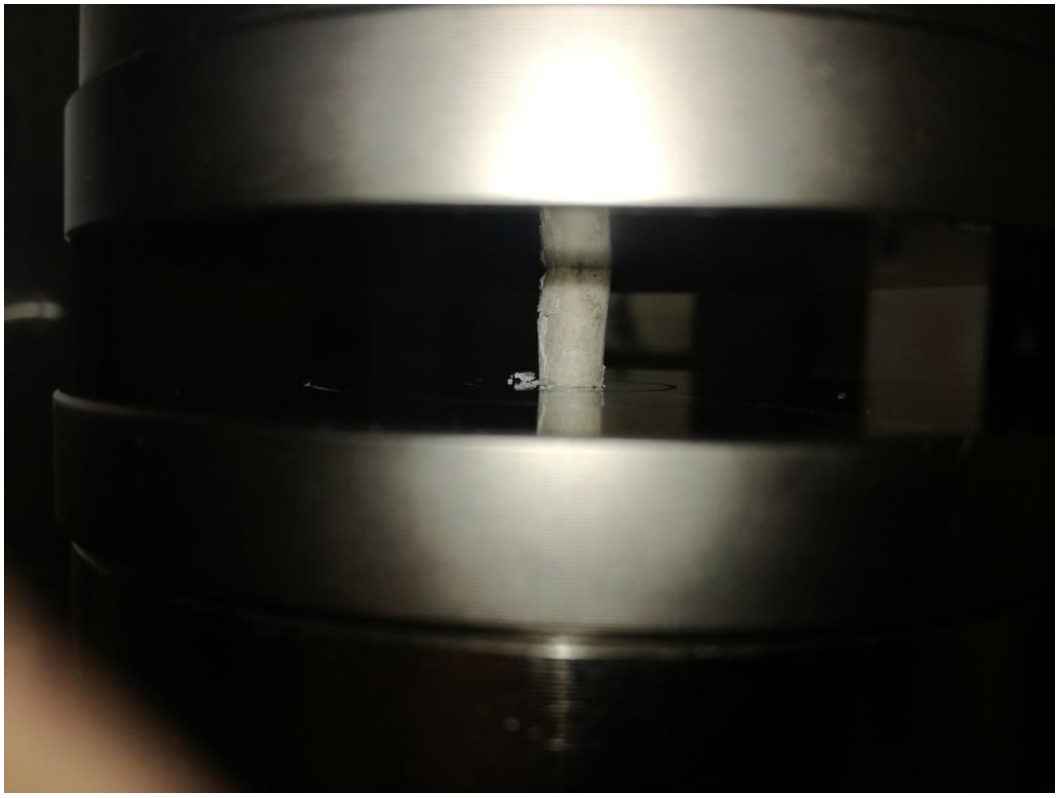

Fig 7: *Mechanical testing of specimen*

Fig 8: *Set of 6 specimens ready to be put in incubator*

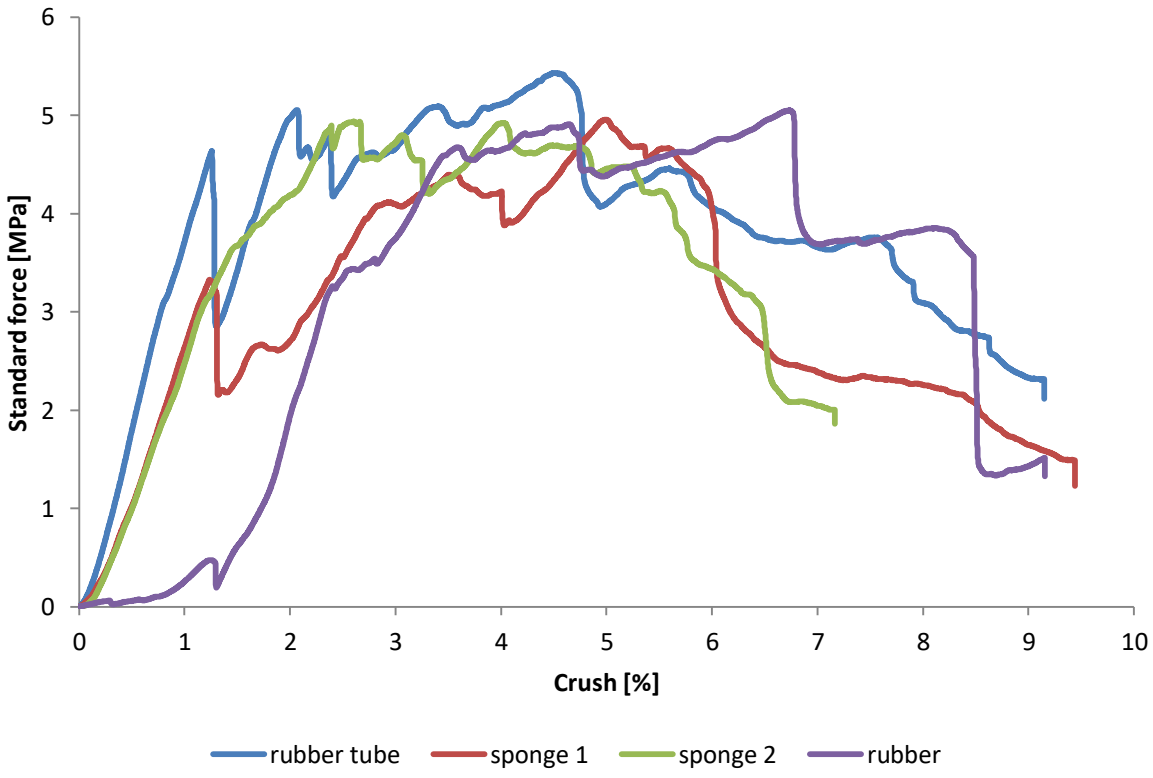

Fig 9: *Graph of results from measuring the compressive strength of cement samples for optimization*

Due to simmilar CS of the 3 types of moulds as optimum solution in optimization process seemed usage of PTFE mold for the reason that PTFE mold was easiest-to-work-with material of the filling process of the optimization phase.

5 RESULTS AND DISCUSSION

5.1 Compresive strength

As shown in fig. 10 most rapid increase in compresive strength represented specimens of cement with activated TCP powder. This could be possible due to more specific area in powder particles. All of the cement types showed decrease in CS between 7 and 14 days of setting time. This could be possible due to lower pH during precipitation and therofere cement matrix and the fibers may degrade.

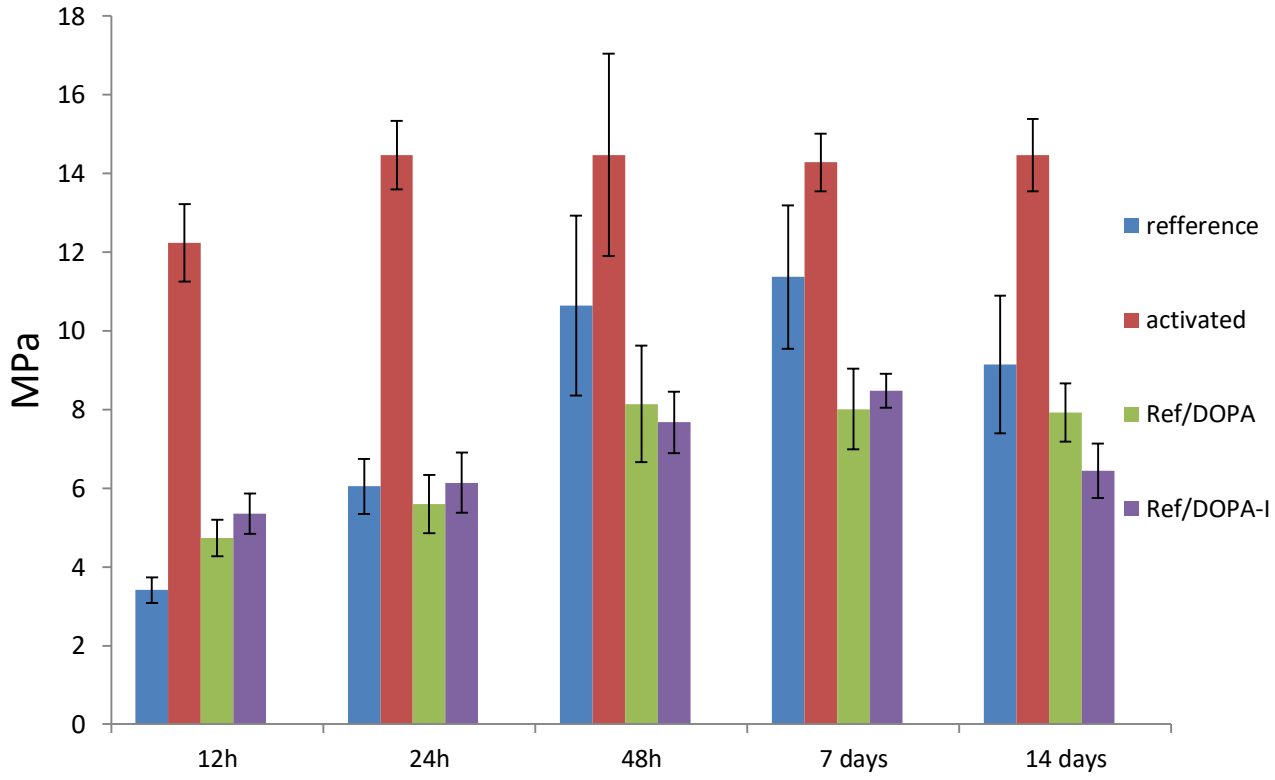

Fig 10: *Graph of results from measuring the compressive strength of cement samples*

5.2 Rheology

The rheological properties (including viscosity and thixotropy) of the injectable pastes were tested with a rheometer (Brookfield, R/S Rheometer). Viscosity (Pa s) is a measurement of a fluid's resistance to flow, describing the internal friction of a moving fluid. Thixotropy stands for the property exhibited by certain gels of becoming fluid when stirred or shaken from semisolid in static and returning to the semisolid state again upon standing. Thixotropy measurements were used to characterize the stability of the pastes. As shown in Fig 11 and Fig 12 cement pastes with Dopamine

and Iodine has better setup time (131 min for Dopamine+Iodine, 216 min for Dopamine) in comparrison with no-modified cement paste (327 min). Simmilar, but higher differnce insetting time represented cement with activated TCP powder in comparrison to cement with reffernced TCP powder (15 min for activated TCP powder and 327 min for non activated TCP powder). This may be possible due to much higher surface area for activated TCP powder). Moreover cement with Iodine and Dopamine showed better manipulation time due to lower storage modulus during the setting time.

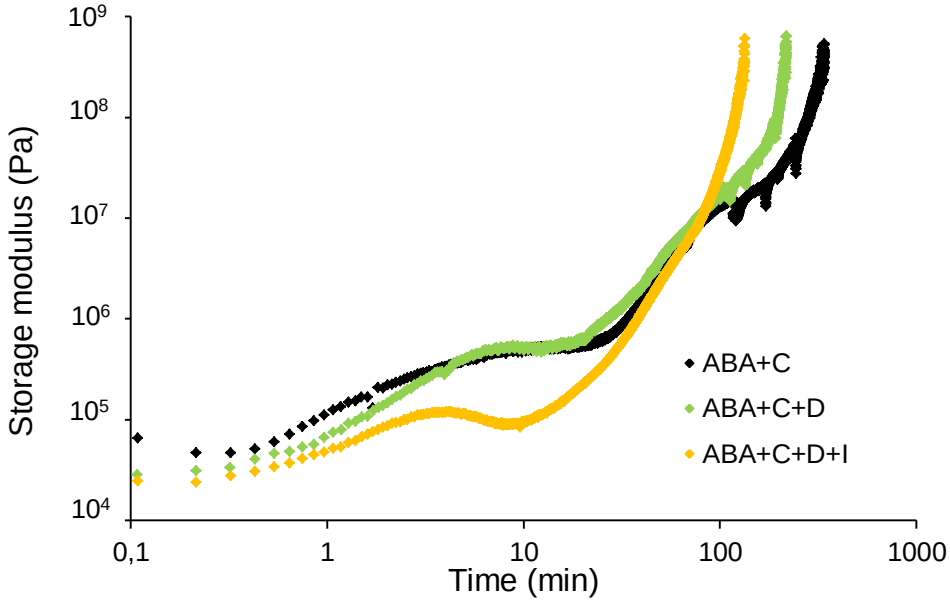

Fig 11: *Rheology graph of.CPCs Paste. Black line represents paste with no adititves.Green represents paste with Dopamine and yellow represents paste with dopamin with iodine.*

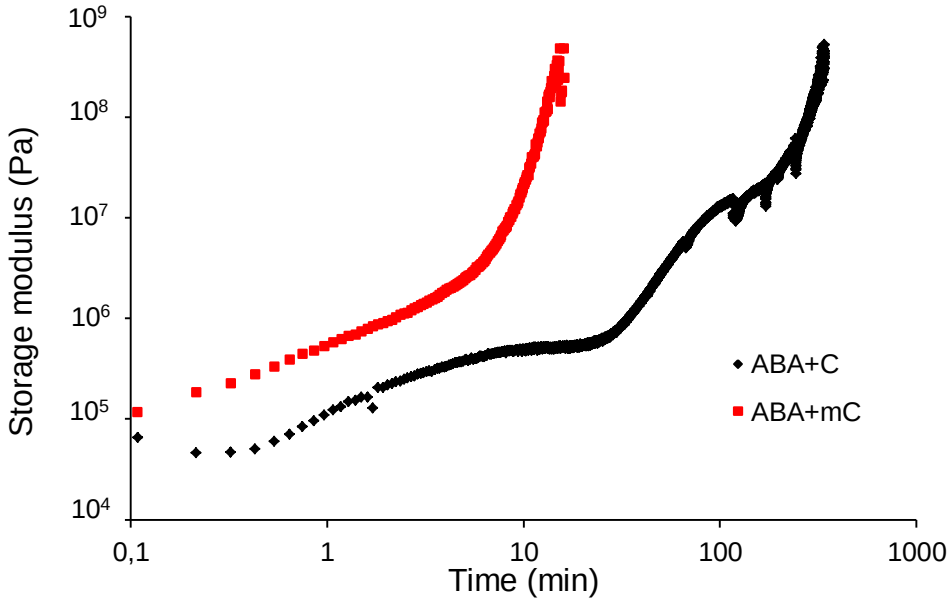

Fig 12: *Rheology graph of.CPCs Paste. Black line represents paste with non-activated TCP and red line represents paste with activated TCP.*

5.3 Crystallinity

X-ray diffraction (XRD) patterns (Fig.8) showed how TCP were converted to the apatite phase after 2 weeks of incubation. Lines represents main 6 peaks of α-TCP. (12.1, 14.02, 15.16, 22.2 and 30.74). During time it can vbe seen that main peaks representing TCP are gradually decreasing which mean that TCP is converted to CDHA over time.

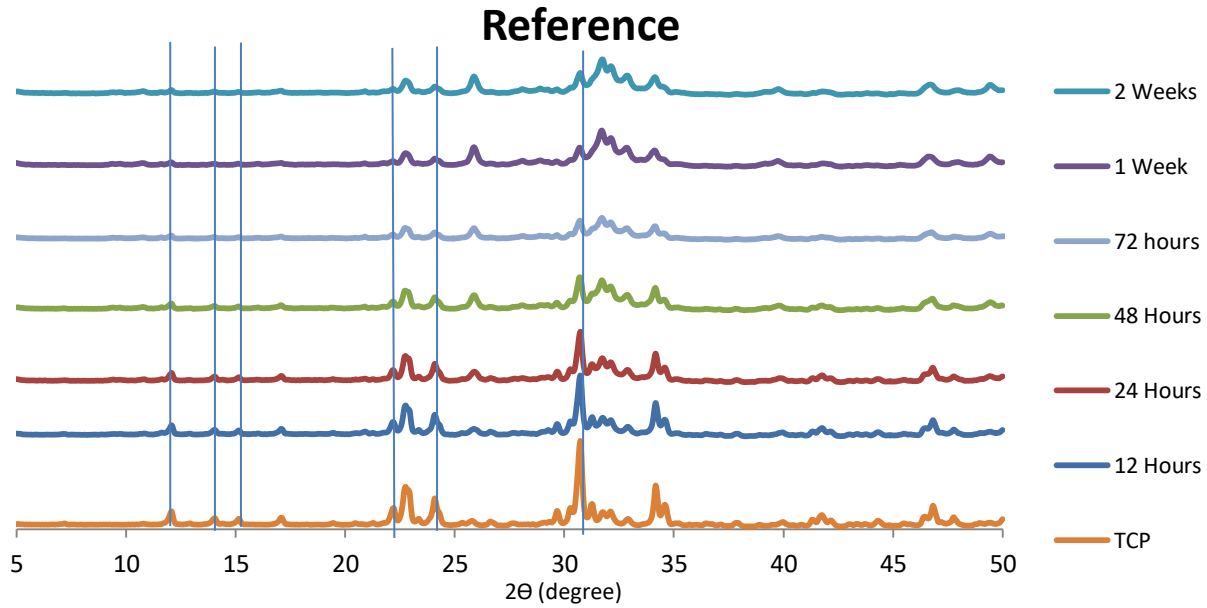

Fig 13: *XRD Graph of conversion TCP to CDHA of cement with reference powder.*

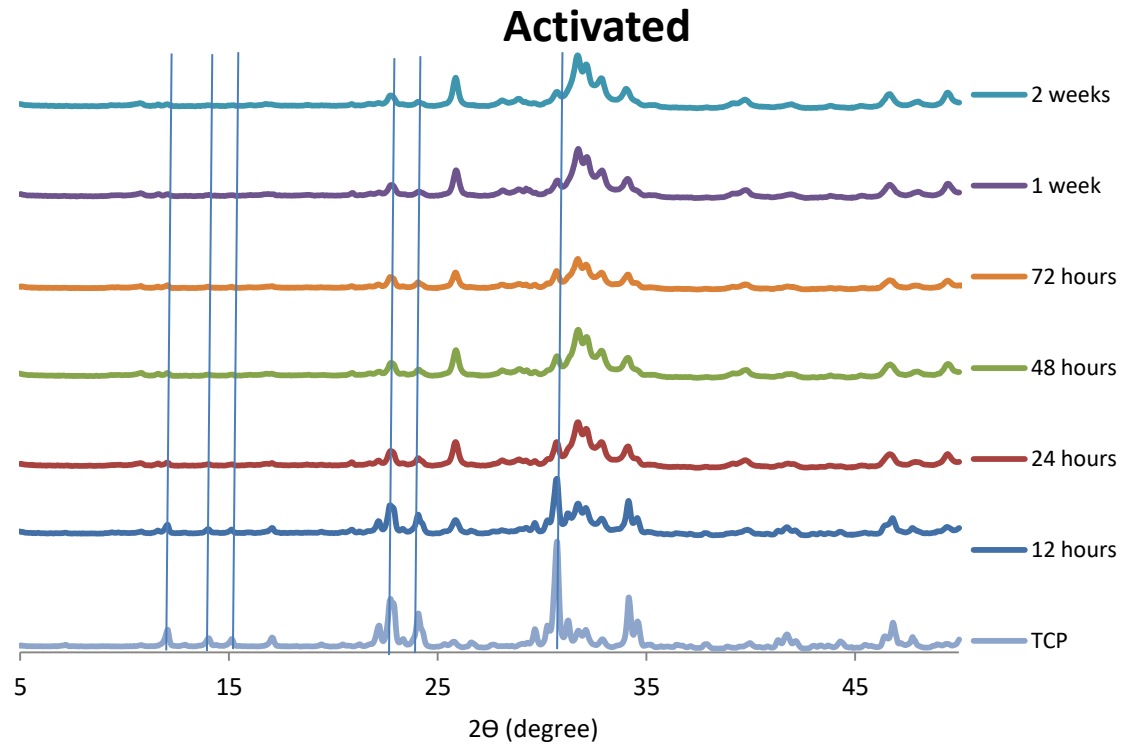

Fig 14: *XRD Graph of conversion TCP to CDHA of cement with milled powder.*

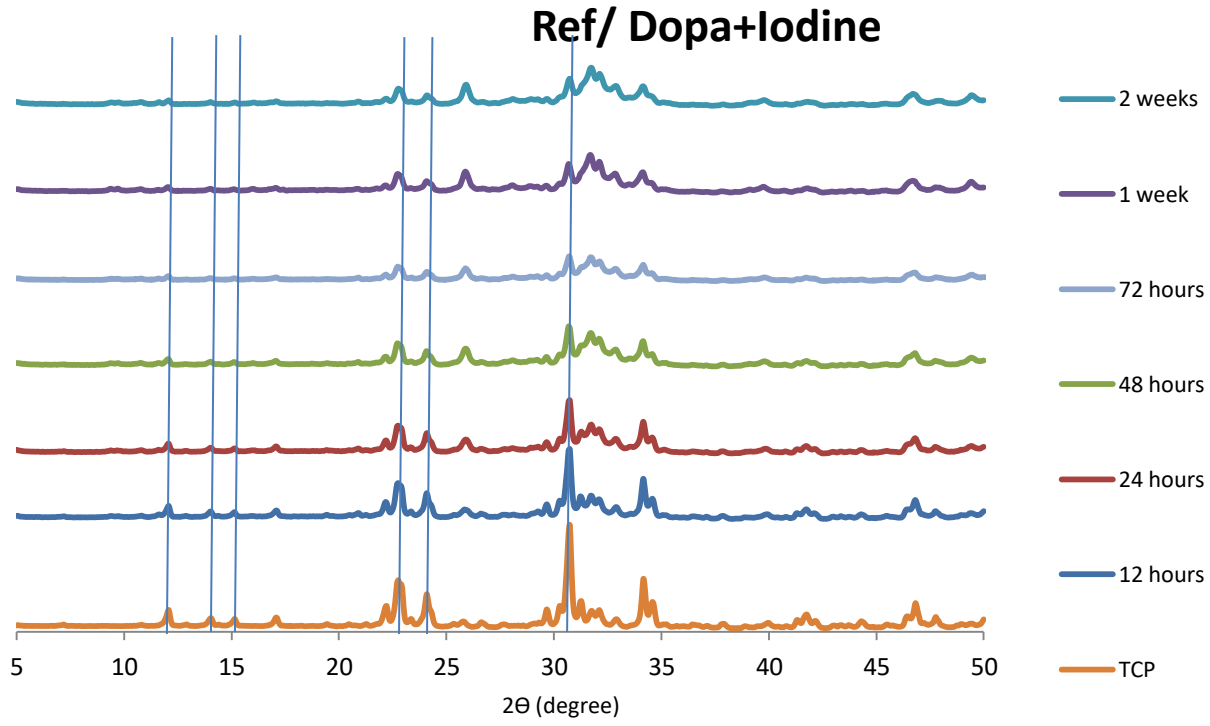

Fig 15: *XRD Graph of conversion TCP to CDHA of cement modified with dopamine and iodine*

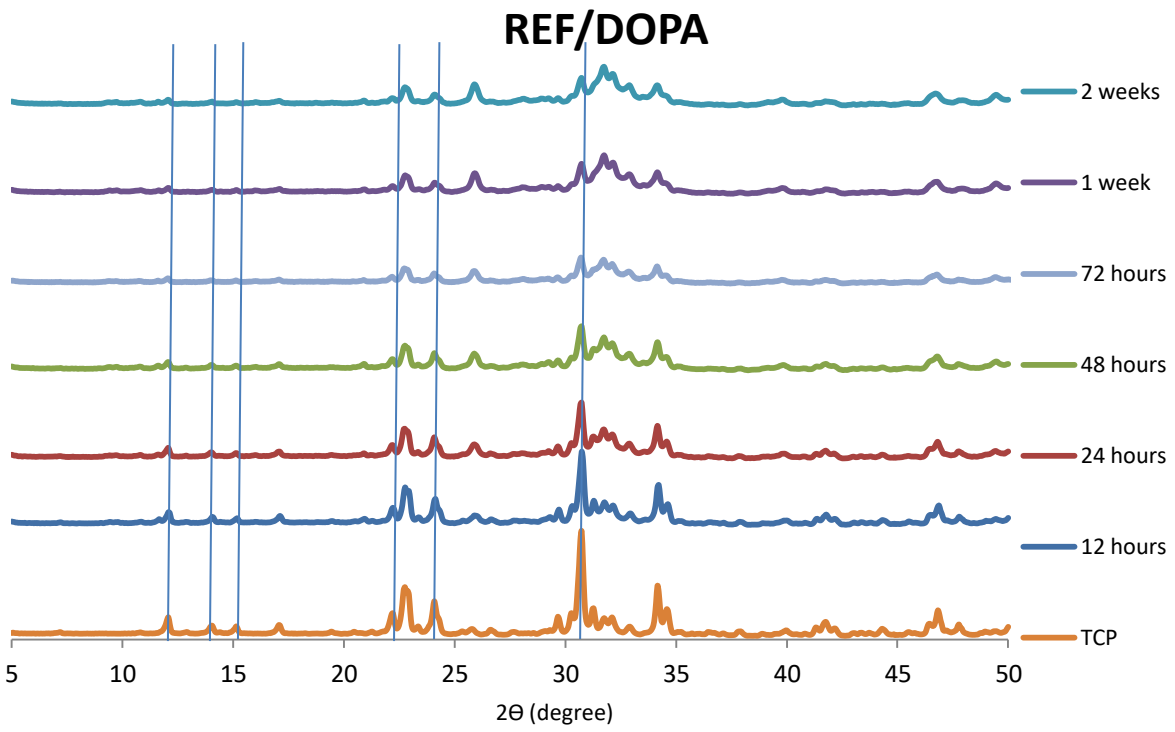

Fig 16: *XRD Graph of conversion TCP to CDHA of cement modified with dopamine*

CPC samples setting kinetics is represented by the transformation of α-TCP to CDHA. Quantification of this process is done by calculation of conversion according to Eq 4. And Eq 5.

$$w_t = \frac{I_t M_{CDHA}}{M_{\alpha-TCP} I_0 - I_t (M_{\alpha-TCP} - M_{CDHA})} \tag{4}$$

$$R_t = \frac{w_0 - w_t}{w_0} * 100 \tag{5}$$

I_t is is intensity of the peak at time t, I_0 is intensity of the peak at zero time of reaction. $M_{\alpha-TCP}$ is adsorption coefficient of α-TCP. (86,43cm^2/g), M_{CDHA} is the adsorption coefficient of CDHA (84,97 cm^2/g), w_0 is the initial fraction of α-TCP in sample =1, w_t is the fraction of α-TCP at time *t* and R_t is the percentage of reaction to time *t*.

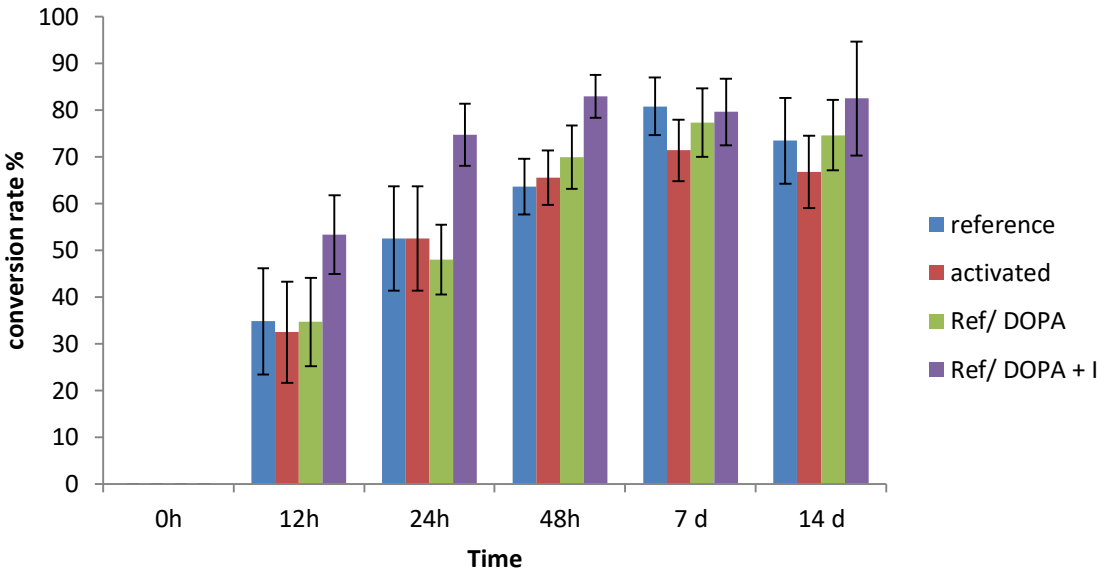

Fig 17: Graph of conversion TCP to CDHA. Bars represent amount of conversion rate TCP to CDHA.

The results from XRD analysis show that most of the TCP is converted to CDHA already after 48 hours of setting. Most rapid conversion have powder with Iodine addition.

5.4 Chemical composition

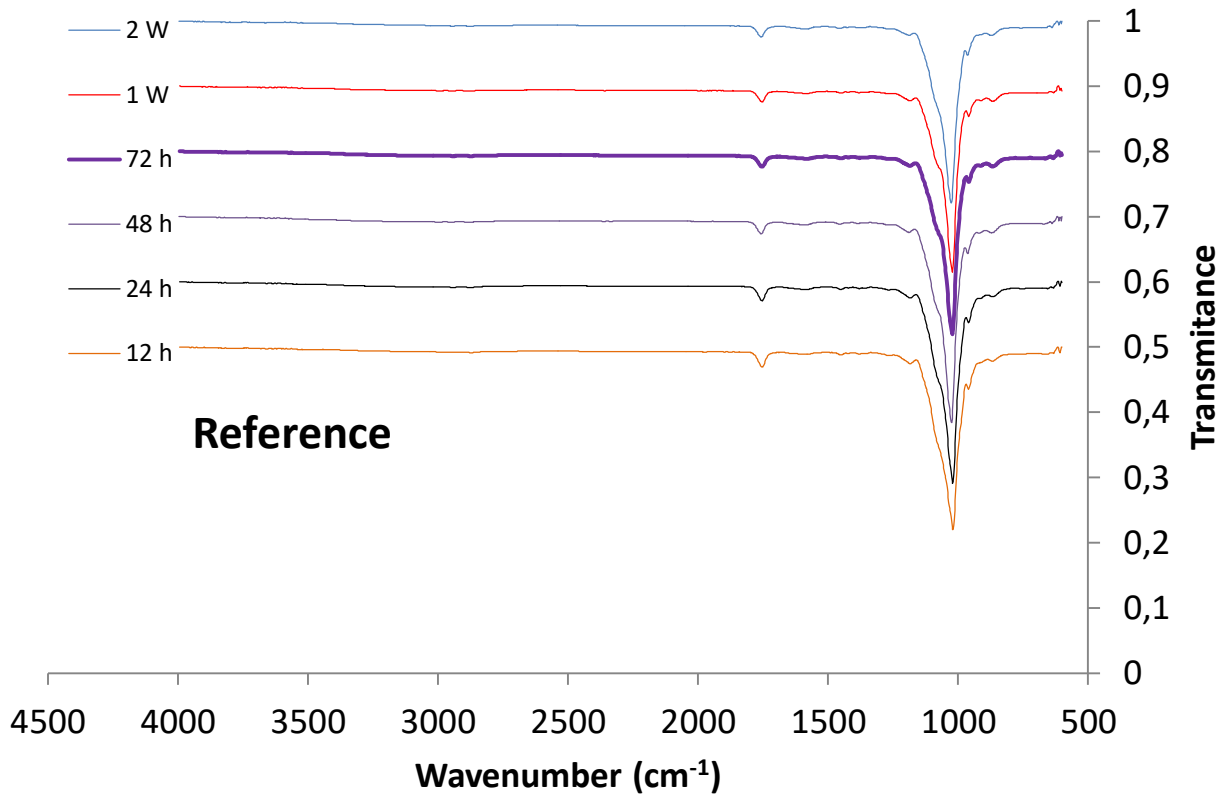

Fig 18: *FTIR graph of referenced TCP powder*

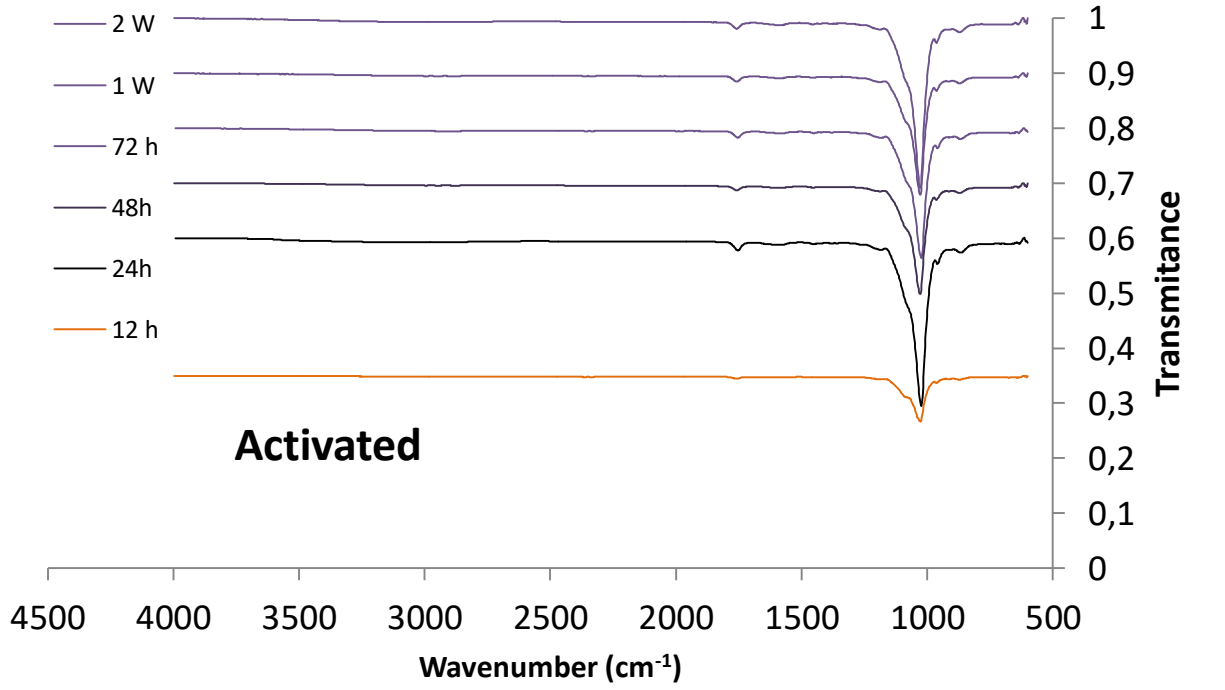

Fig 19: *FTIR graph of cement speciment prepared with milled TCP powder*

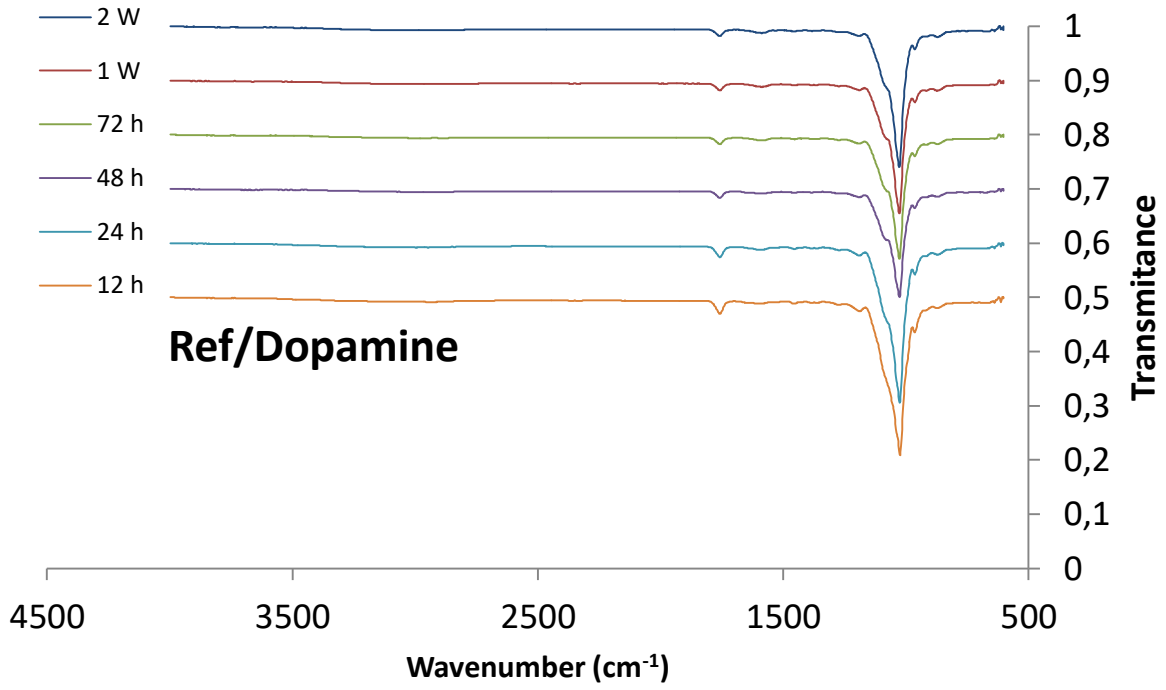

Fig 19: *FTIR graph of TCP powder modified with Dopamine*

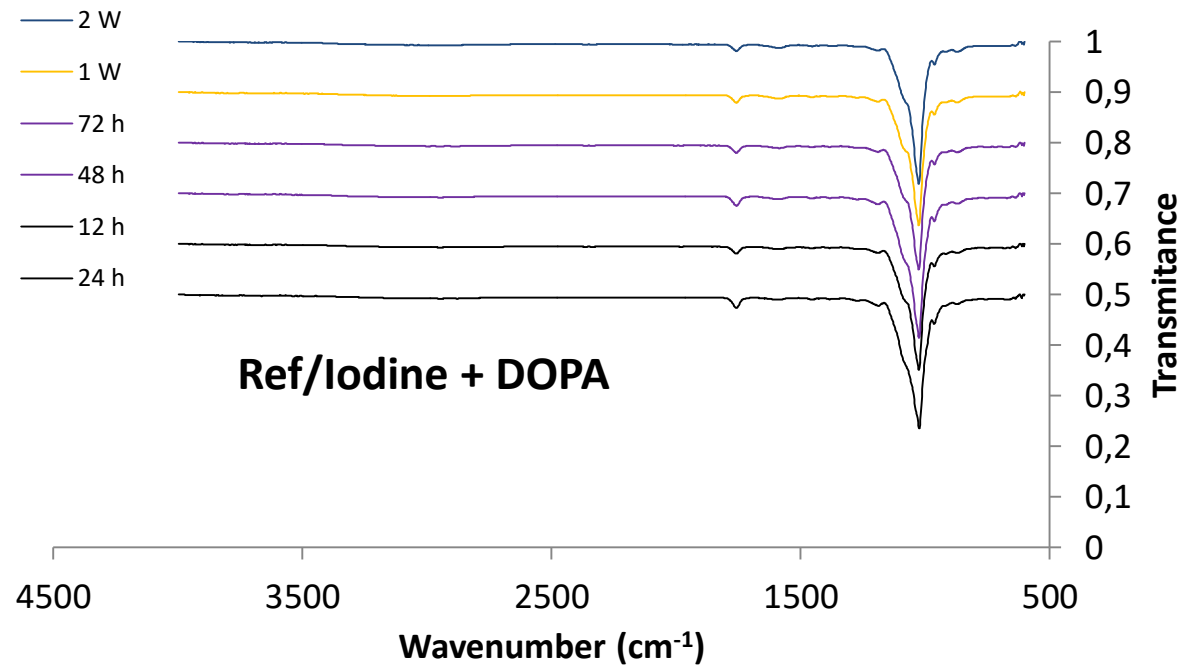

Fig 20: *FTIR graph of TCP powder modified with Iodine + Dopamine*

Cement samples with additives and without additives were compared using ATR-FTIR method. There are no differnces between spectra. Chemical bonding in the material is not affected during conversion process.

5.5 Morphology

SEM micrographs of the fractured surfaces after mechanical testing were analyzed and the representative images of setting time 12 hours and 1 week were compared.

Reference TCP powder

12 hours

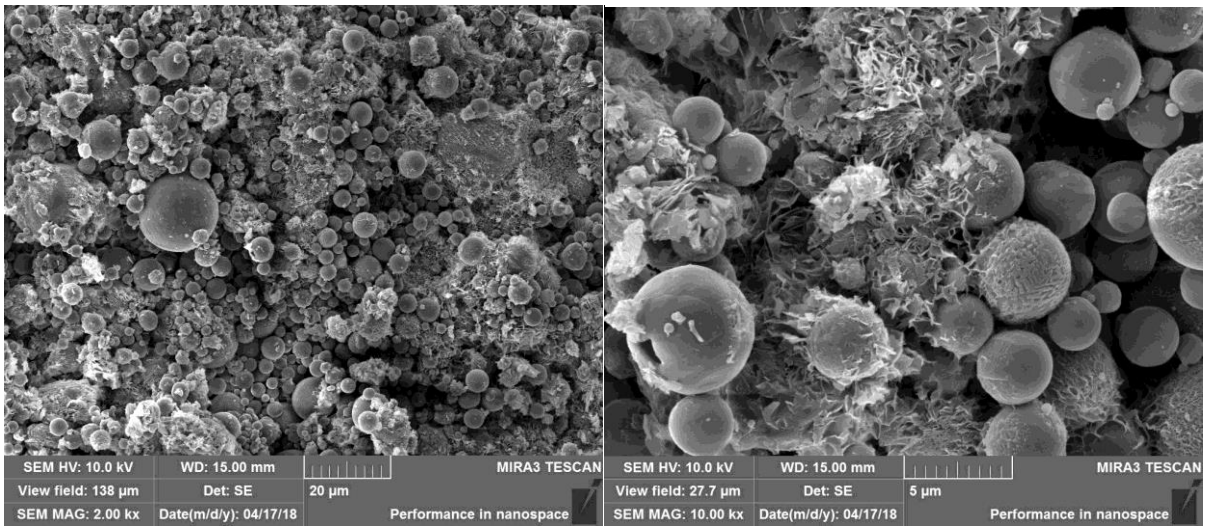

Fig 21: *SEM image of cement prepared with no activated TCP powder magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 12 hours*

1 week

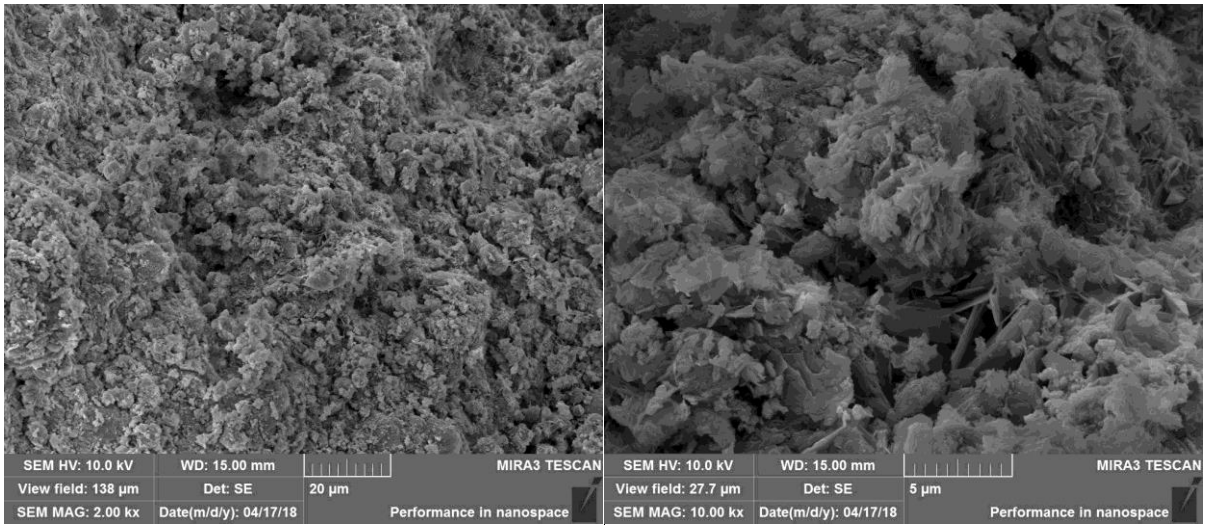

Fig 22: *SEM image of cement prepared with no activated TCP powder magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 1 Week*

Activated TCP powder

12 hours

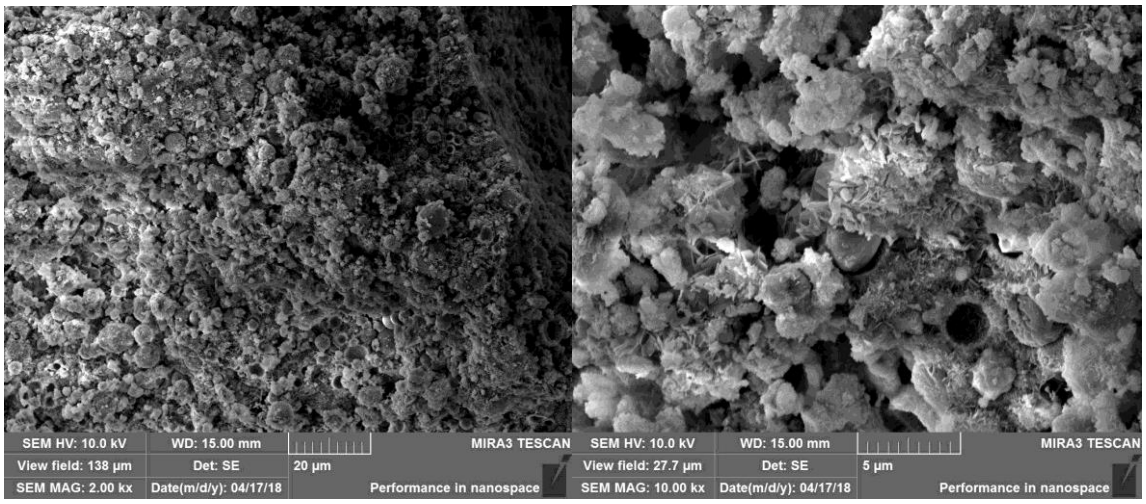

Fig 23: *SEM image of cement prepared with activated TCP powder magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 12 hours*

1 week

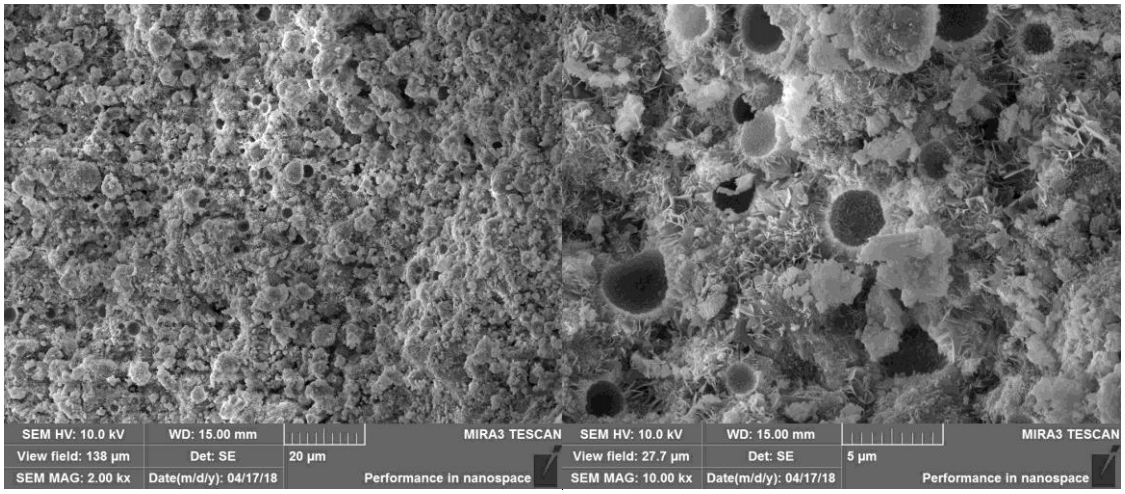

Fig 24: *SEM image of cement prepared with activated TCP powder magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 1 week*

TCP powder with DOPA

12 hours

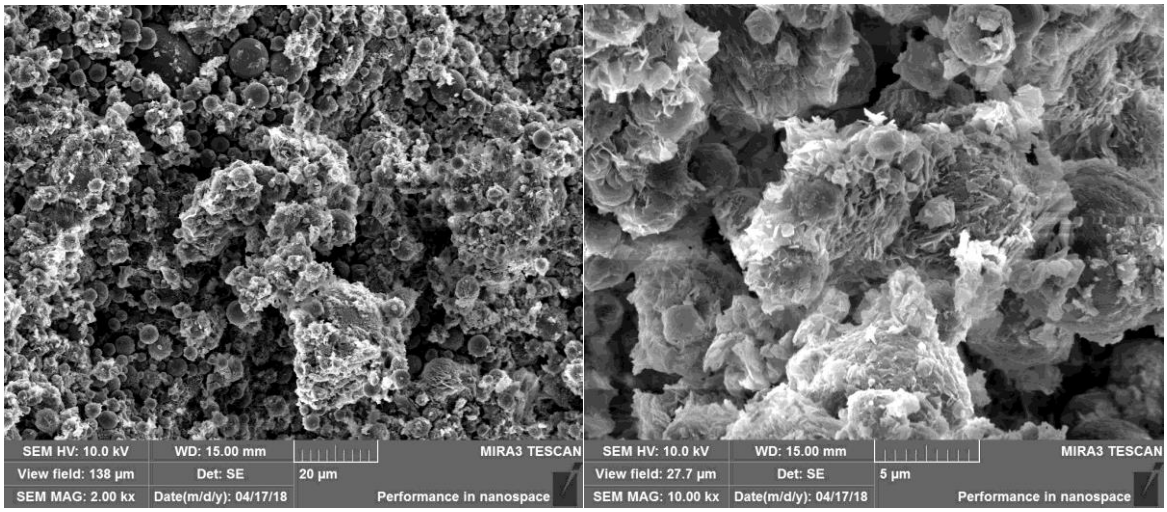

Fig 25: *SEM image of cement prepared with no activated TCP powder with DOPA magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 12 hours*

1 week

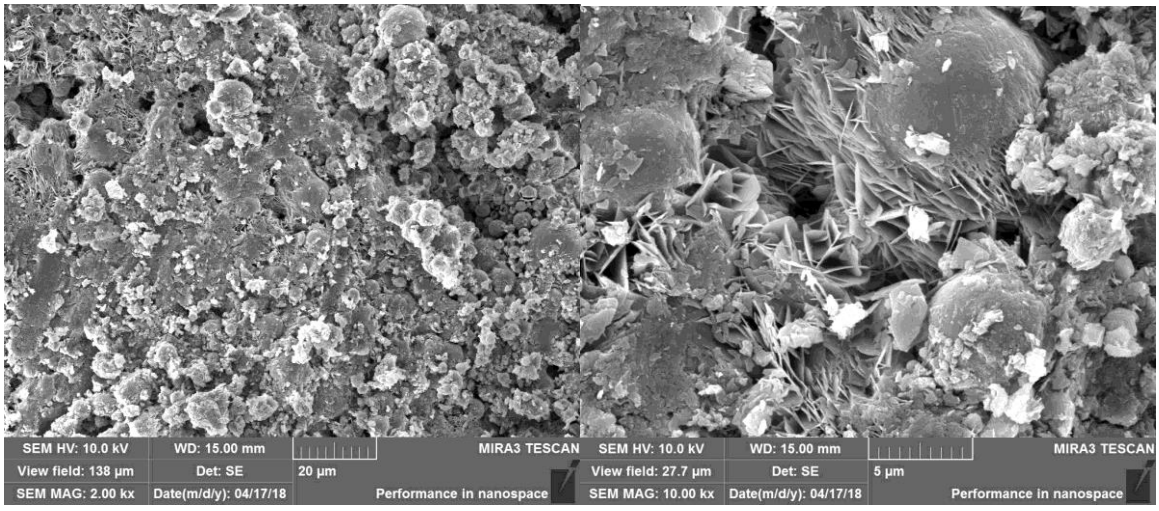

Fig 26: *SEM image of cement prepared with no activated TCP powder with DOPA by 2Kx zoom on left and 10kx zoom on right set for hardenning for 1 week*

TCP powder with DOPA + $NaIO_3$

12 hours

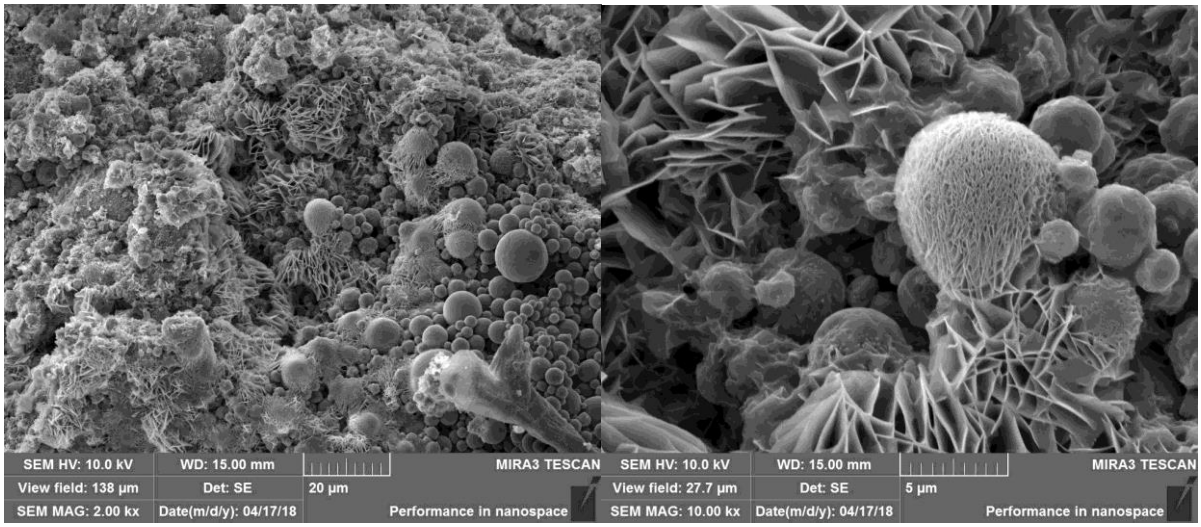

Fig 27: *SEM image of cement prepared with no activated TCP powder with DOPA+ Iodine magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 12 hours*

1 week

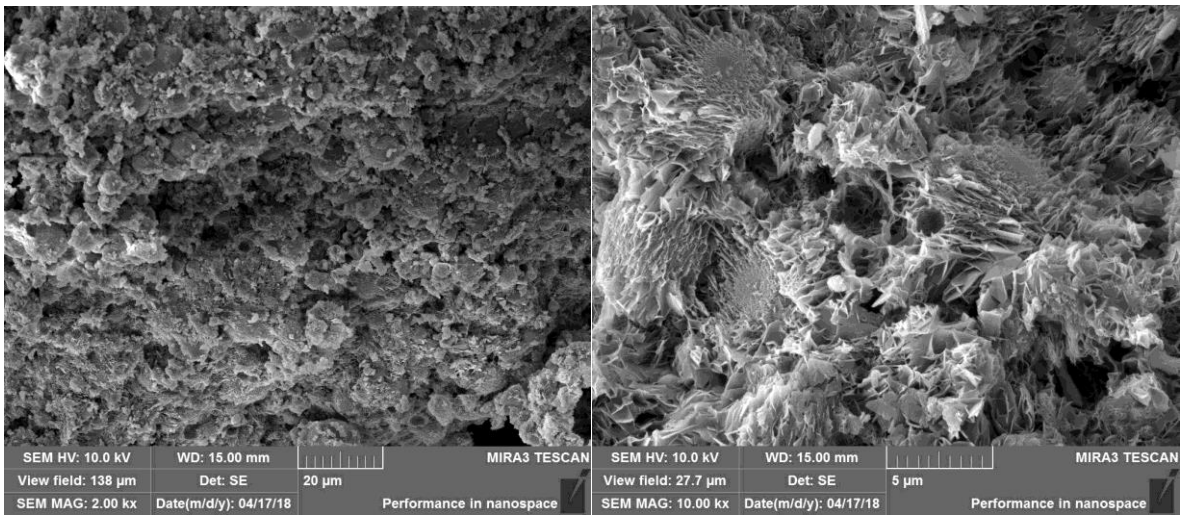

Fig 28: *SEM image of cement prepared with no activated TCP powder with DOPA+ Iodine magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 1 week*

6 CONCLUSION

This work deals with preparation and characterization of samples from composite calcium cement based calcium phosphate (CPC). The theoretical part describes CPCs as a bone repair material, the properties and structure of calcium phosphates, including their limitations to medicinal application.in the experimental part is described the determination of sample preparation technique using ultrapure water experiments. As a optimum technique involves using a PTFE mold, terminating curing reactions with absolute cold ethanol to remove water from specimens, and drying the samples in a vacuum oven. A morphological study using scanning electron microscopy (SEM) was performed for times 12 hours and 1 week due to be able to compare change in their crystalline structure, which was also examined by X-ray diffraction (XRD) to determine α-phosphate conversion calcium to calcium deficient hydroxyapatite (CDHA). XRD, SEM and Mechanical analysis showed that most of the conversion α-tricalcium phosphate to calcium deficient hydroxyapatite (CDHA) is done within first 48 hours from seting. FTIR analysis confirmed that by setting phase no new bonds rises. Most of the mechanical properties of CPC samples were examined by mechanical compression tests. The test results showed stable strength of cement specimens prepared with activated TCP powder. However britleness is still need to be imroved. The results of the work show a significant effect on setting time and compresive strength by using activated TCP powder for preparing CPCs on the properties of CPC bone cements. Cements modified with Iodine and Dopamine showed decreasing setting time in comparisson with no modified no activated CPC cements. More work is need to be done to asses this properties for modified CPC cements with activated TCP powder.

7 REFERENCES

[1] CHOW, Laurence. Next generation calcium phosphate-based biomaterials. *Dental Materials Journal* [online]. **2009**, 1-10 [cit. 2017-12-8]. Available from: https://www.jstage.jst.go.jp/article/dmj/28/1/28_1_1/_pdf

[2] BOHNER, M. Design of ceramic-based cements and putties for bone graft substitution. *European Cells and Materials* [online]. 2010, **20**, 1-12 [cit. 2017-12-8]. DOI: 10.22203/eCM.v020a01. Available from: http://ecmjournal.org/journal/papers/vol020/pdf/v020a01.pdf

[3] AJAXON, Ingrid, Anders HOLMBERG, Caroline ÖHMAN-MÄGI a Cecilia PERSSON. Fatigue performance of a high-strength, degradable calcium phosphate bone cement. *Journal of the Mechanical Behavior of Biomedical Materials* [online]. Elsevier, 2017, [cit. 2017-12-8]. Available: https://doi.org/10.1016/j.jmbbm.2017.12.005

[4] AJAXON, Ingrid, Alice ACCIAIOLI, Caroline ÖHMAN-MÄGI, Cecilia PERSSON, Giacomo LIONELLO, Maria-Pau GINEBRA a Massimiliano BALEANI. Elastic properties and strain-to-crack-initiation of calcium phosphate bone cements: Revelations of a high-resolution measurement technique. *Journal of the Mechanical Behavior of Biomedical Materials* [online]. Elsevier, 2017, **74**, 428-437 [cit. 2017-12-8]. Available from: https://doi.org/10.1016/j.jmbbm.2017.06.023

[5] *Biomaterials for Bone Regeneration* [online]. Elsevier, 2014, **74** [cit. 2017-12-8]. ISBN 9780857098047. Available: http://linkinghub.elsevier.com/retrieve/pii/B9780857098047500029

[6] AN, Jie, Joop G. C. WOLKE, John A. JANSEN, Sander C. G. LEEUWENBURGH, Giacomo LIONELLO, Maria-Pau GINEBRA a Massimiliano BALEANI. Influence of polymeric additives on the cohesion and mechanical properties of calcium phosphate cements. *Journal of Materials Science: Materials in Medicine* [online]. Elsevier, 2016, **27**(3), - [cit. 2017-12-8]. DOI: 10.1007/s10856-016-5665-x. ISBN 9780857098047. ISSN 0957-4530. Available from: http://link.springer.com/10.1007/s10856-016-5665-x

[7] PUJARI-PALMER, Michael, Celin,r e ROBO, Sander C. G. LEEUWENBURGH, Giacomo LIONELLO a Håkan ENGQVIST. Influence of cement compressive strength and porosity on augmentation performance in a model of orthopedic screw pull-out. *Journal of the Mechanical Behavior of Biomedical Materials* [online]. Elsevier, 2017, **77**(3), 624-633 [cit. 2017-12-8]. ISBN 9780857098047. Available from: https://doi.org/10.1016/j.jmbbm.2017.10.016 [8]

[8] MEHDIZADEH, Mohammadreza, Hong WENG, Dipendra GYAWALI, Liping TANG a Jian YANG. Injectable citrate-based mussel-inspired tissue bioadhesives with high wet strength for sutureless wound closure. *Biomaterials* [online]. Elsevier, 2012, **33**, 624-633 [cit. 2017-12-8]. DOI: 10.1016/j.biomaterials.2012.07.055. ISBN 9780857098047. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0142961212008502

[9] MENG, Dan, Limin DONG, Ying WEN, Qiufei XIE a Jian YANG. Effects of adding resorbable chitosan microspheres to calcium phosphate cements for bone regeneration. *Materials Science and Engineering: C* [online]. Elsevier, 2015, **47**, 266-272 [cit. 2017-12-8].

DOI: 10.1016/j.msec.2014.11.049. ISBN 9780857098047. ISSN 09284931. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0928493114007504

[10] ARYAEI, Ashkan, Jason LIU, Ahalapitiya.H. JAYATISSA, A. CHAMPA JAYASURIYA a Jian YANG. Cross-linked chitosan improves the mechanical properties of calcium phosphate–chitosan cement. *Materials Science and Engineering: C* [online]. Elsevier, 2015, **54**, 14-19 [cit. 2017-12-8]. DOI: 10.1016/j.msec.2015.04.024. ISBN 9780857098047. ISSN 09284931. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0928493115300254

[11] LUO, Jun, Ingrid AJAXON, Maria Pau GINEBRA, Håkan ENGQVIST and Cecilia PERSSON. Compressive, diametral tensile and biaxial flexural strength of cutting-edge calcium phosphate cements. *Journal of the Mechanical Behavior of Biomedical Materials* [online]. 2016, July 2016, (60), 617-627 [cit. 2018-01-20]. ISSN 1751-6161. Available from: http://www.sciencedirect.com/science/article/pii/S1751616116300480#f0005

[12] MISTRY A,Mikos A. Tissue Engineering Strategiesfor BoneR egeneration.In:Yannas I, editor. Regenerative Medicine II: Springer Berlin Heidelberg; 2005. p. 1-22

[13] DOBLARÉ M, Garcıa JM, Gómez MJ. Modelling bone tissue fracture and healing: a review. Engineering Fracture Mechanics 2004;71:1809-40.

[14] MANOLAGAS SC. Birth and Death of Bone Cells:Basic Regulatory Mechanisms and Implications for the Pathogenesis and Treatment of Osteoporosis. Endocrine Reviews 2000;21:115-37.

[15] ERIKSEN E. Cellular mechanisms of bone remodeling. Reviews in Endocrine and Metabolic Disorders 2010;11:219-27.

[16] CAPULLI M, Paone R, Rucci N. Osteoblast and osteocyte: Games without frontiers. Archives of Biochemistry and Biophysics 2014;561:3-12

[17] KARTSOGIANNIS V, Ng KW. *Cell lines and primary cell cultures in the study of bone cell biology*. Molecular and Cellular Endocrinology 2004;228:79-102.

[18] BONEWALD LF. The amazing osteocyte. Journal of Bone and Mineral Research 2011;26:229-38

[19] MONTAZEROLGHAEM, Maryam. *Additives Increasing the Bone-Forming Potential around Calcium Phosphate Cements: Statin, Strontium and Silicon*. 2015. ISBN 9789155491833. ISSN 1651-6214.

[20] DOROZHKIN, S.V. Self-Setting Calcium Orthophosphate Formulations. *J. Funct. Biomater.* **2013**, *4*, 209-311. Available from: http://www.mdpi.com/2079-4983/4/4/209

[21] JACK, V F J Buchanan, and N J Dunne Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine Vol 222, Issue 1, pp. 19 - 28 First Published February 26, 2008 Available from: https://doi.org/10.1243/09544119JEIM312

[22] GINEBRA, Maria-Pau, Cristina CANAL, Montserrat ESPANOL, David PASTORINO a Edgar B. MONTUFAR. Calcium phosphate cements as drug delivery materials. *Advanced Drug Delivery Reviews* [online]. Elsevier B.V, 2012, **64**(12), 1090-1110 [cit. 2018-05-14]. DOI: 10.1016/j.addr.2012.01.008. ISSN 0169-409X. [12]

[23] GINEBRA, M.P. Calcium phosphate bone cements. *Orthopaedic Bone Cements*. Elsevier, 2008, s. 206-230. DOI: 10.1533/9781845695170.2.206. ISBN 9781845693763.

[24] O. Critical review: Injectability of calcium phosphate pastes and cements. *Acta Biomaterialia* [online]. Elsevier, 2017, **50**, 1-19 [cit. 2018-05-14]. DOI: 10.1016/j.actbio.2016.11.019. ISSN 1742-7061.

[25] CHOW L.C., E.D. Eanes Calcium phosphate cement. Octacalcium phosphate Karger,Basel (2001)p. 148–163

[26] XU, Hockin H. K. a Carl G. SIMON. Self-hardening calcium phosphate cement–mesh composite: Reinforcement, macropores, and cell response. *Journal of Biomedical Materials Research Part A* [online]. Hoboken: Wiley Subscription Services, Inc., A Wiley Company, 2004, **69**(2), 267-278 [cit. 2018-05-14]. DOI: 10.1002/jbm.a.20124. ISSN 1549-3296.

[27] ZHANG, Jingtao, Weizhen LIU, Verena SCHNITZLER, Franck TANCRET a Jean-Michel BOULER. Calcium phosphate cements for bone substitution: Chemistry, handling and mechanical properties. *Acta Biomaterialia* [online]. Elsevier, 2014, **10**(3), 1035-1049 [cit. 2018-05-14]. DOI: 10.1016/j.actbio.2013.11.001. ISSN 1742-7061.

[28] DURUCAN, C. a P.W. BROWN. Kinetic model for α-tricalcium phosphate hydrolysis. *Journal of the American Ceramic Society* [online]. Blackwell Publishing, 2002, **85**(8), 2013-2018 [cit. 2018-05-14]. DOI: 10.1111/j.1151-2916.2002.tb00397.x. ISSN 00027820.

[29] GBURECK, Uwe, Jake E. BARRALET, Lavinia RADU, Hans G. KLINGER a Roger THULL. Amorphous α-Tricalcium Phosphate: Preparation and Aqueous Setting Reaction. *Journal of the American Ceramic Society* [online]. Westerville, Ohio: American Ceramics Society, 2004, **87**(6), 1126-1132 [cit. 2018-05-14]. DOI: 10.1111/j.1551-2916.2004.01126.x. ISSN 0002-7820.

[30] GINEBRA, M., E. FERNÁNDEZ, F. DRIESSENS, M. BOLTONG, J. MUNTASELL, J. FONT a J. PLANELL. The effects of temperature on the behaviour of an apatitic calcium phosphate cement. *Journal of Materials Science: Materials in Medicine* [online]. Dordrecht: Kluwer Academic Publishers, 1995, **6**(12), 857-860 [cit. 2018-05-14]. DOI: 10.1007/BF00134332. ISSN 0957-4530.

[31] TENHUISEN, K. a P. BROWN. The effects of citric and acetic acids on the formation of calcium-deficient hydroxyapatite at 38 °C. *Journal of Materials Science: Materials in Medicine* [online]. Dordrecht: Kluwer Academic Publishers, 1994, **5**(5), 291-298 [cit. 2018-05-14]. DOI: 10.1007/BF00122399. ISSN 0957-4530.

[32] GINEBRA, M.P., F.C.M. DRIESSENS a J.A. PLANELL. Effect of the particle size on the micro and nanostructural features of a calcium phosphate cement: a kinetic analysis. *Biomaterials* [online]. Elsevier, 2004, **25**(17), 3453-3462 [cit. 2018-05-14]. DOI: 10.1016/j.biomaterials.2003.10.049. ISSN 0142-9612.

[33] SCHNITZLER, Verena, Franck FAYON, Christelle DESPAS, et al. Investigation of alendronate-doped apatitic cements as a potential technology for the prevention of osteoporotic hip fractures: Critical influence of the drug introduction mode on the in vitro cement properties. *Acta Biomaterialia* [online]. Elsevier, 2011, **7**(2), 759-770 [cit. 2018-05-14]. DOI: 10.1016/j.actbio.2010.09.017. ISSN 1742-7061.

[34] DESPAS, Christelle, Verena SCHNITZLER, Pascal JANVIER, Franck FAYON, Dominique MASSIOT, Jean-Michel BOULER, Bruno BUJOLI a Alain WALCARIUS. High-frequency impedance measurement as a relevant tool for monitoring the apatitic cement setting reaction. *Acta Biomaterialia* [online]. Elsevier, 2014, **10**(2), 940-950 [cit. 2018-05-14]. DOI: 10.1016/j.actbio.2013.10.019. ISSN 1742-7061.

[35] Hofmann M.P., A.M. YOUNG, U. GBURECK, S.N. NAZHAT, J.E. BARRALET FTIR-monitoring of a fast setting brushite bone cement: effect of intermediate phasesJ Mater Chem, 16 (2006), pp. 3199-3206

[36] FERNANDEZ, E, M GINEBRA, M BOLTONG, Fcm DRIESSENS, J GINEBRA, Eap DE MAEYER, Rmh VERBEECK a Ja PLANELL. Kinetic study of the setting reaction of a calcium phosphate bone cement. *Journal of Biomedical Materials Research, Part B: Applied Biomaterials* [online]. 1996, **32**(3), 367-374 [cit. 2018-05-14]. DOI: 10.1002/(SICI)1097-4636(199611)32:3<367::AID-JBM9>3.0.CO;2-Q. ISSN 0021-9304.

[37] BOHNER, Marc, Reto LUGINBÜHL, Christian REBER, Nicola DOEBELIN, Gamal BAROUD a Egle CONFORTO. A physical approach to modify the hydraulic reactivity of α-tricalcium phosphate powder. *Acta Biomaterialia* [online]. Elsevier, 2009, **5**(9), 3524-3535 [cit. 2018-05-14]. DOI: 10.1016/j.actbio.2009.05.024. ISSN 1742-7061.

[38] M. BohnerReactivity of calcium phosphate cements J Mater Chem, 17 (2007), pp. 3980-3986 http://pubs.rsc.org/en/Content/ArticleLanding/2007/JM/b706411j#!divAbstract

[39] BOHNER, M., U. GBURECK a J.E. BARRALET. Technological issues for the development of more efficient calcium phosphate bone cements: A critical assessment. *Biomaterials* [online]. Elsevier, 2005, **26**(33), 6423-6429 [cit. 2018-05-14]. DOI: 10.1016/j.biomaterials.2005.03.049. ISSN 0142-9612.

[40] Moreau, J.L.; Weir, M.D.; Xu, H.H.K. Self-setting collagen-calcium phosphate bone cement: Mechanical and cellular properties. J. Biomed. Mater. Res. A 2009, 91, 605–613.

[41] Chamel, Martha & Groll, Jürgen & Gbureck, Uwe. (2015). Reinforcement Strategies for Load-Bearing Calcium Phosphate Biocements. Materials. 8. 2700. 10.3390/ma8052700. Available from: https://www.researchgate.net/publication/276963558_Reinforcement_Strategies_for_Load-Bearing_Calcium_Phosphate_Biocements/citation/download

8 LIST OF ABBREVIATIONS

CPCs calcium phosphate cements

HA hydroxyapatite

TTCP tetracalcium phosphate

DCPD dicalcium phosphate dihydrate

FDA food and drug administration

CDHA calcium deficient hydroxyapatite

TRIS tris(hydroxymethyl)aminometan

DOPA dihydroxyphenylalanine

MCP monocalcium phosphate

TCP tricalcium phosphate

CS compressive strength

PTFE polytereftalate

MSC mesenchymal

9 LIST OF FIGURES

Fig 1: *The illustration shows the structures of cortical bone, which is composed of cylindrical-shaped osteons arranged around Haversian canals containing blood vessels*

Fig 2: *Osteoclasts are derived from monocyte/macrophage cell lineage and re-sorb bone matrix, while osteoblasts are derived from MSCs and produce new bone matrix. The osteocytes are embedded within the bone matrix and are responsible for signal transduction*

Fig 3: *The cement sets as a result of a dissolution and precipitation process* [23]

Fig 4: *Classification of calcium phosphate cements, with examples of the most common formulations. From top to bottom the cements are classified by the type of end-product(apatite or brushite), number of components in the solid phase (single or multiple), type of setting reaction (hydrolysis or acid–base reaction), setting mechanism and microstructure evolution during setting. Scanning electron micrographs of set apatite and brushite cements obtained by the hydrolysis of α tricalcium phosphate (α-TCP) and by reaction of β-TCP with MCPM (monocalcium phosphate monohydrate) respectively, are also shown. [22]*

Fig 5: 3 *Types of moulds (rubber, sponge, rubber tube) used as forms filled with cement paste*

Fig 6: *Cylindric shaped sample of hardened cement*

Fig 7: *Mechanical testing of specimen*

Fig 8: *Set of 6 specimens ready to be put in incubator*

Fig 9: *Graph of results from measuring the compressive strength of cement samples for optimization*

Fig 10: *Graph of results from measuring the compressive strength of cement samples*

Fig 11: *Rheology graph of.CPCs Paste. Black line represents paste with no adititves.Green represents paste with Dopamine and yellow represents paste with dopamin with iodine*

Fig 12: *Rheology graph of.CPCs Paste. Black line represents paste with non-activated TCP and red line represents paste with activated TCP.*

Fig 13: *XRD Graph of conversion TCP to CDHA of cement with reference powder*

Fig 14: *XRD Graph of conversion TCP to CDHA of cement with milled powder*

Fig 15: *XRD Graph of conversion TCP to CDHA of cement modified with dopamine*

Fig 16: *XRD Graph of conversion TCP to CDHA of cement modified with dopamine and iodine*

Fig 17: *Graph of conversion TCP to CDHA Bars represent amount of conversion rate TCP to CDHA.*

Fig 18: *FTIR graph of referenced TCP powder*

Fig 19: *FTIR graph of TCP powder modified with Dopamine*

Fig 20: *FTIR graph of TCP powder modified with Iodine + Dopamine*

Fig 21: *SEM image of cement prepared with no activated TCP powder magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 12 hours*

Fig 22: *SEM image of cement prepared with no activated TCP powder magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 1 Week*

Fig 23: *SEM image of cement prepared with activated TCP powder magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 12 hours*

Fig 24: *SEM image of cement prepared with activated TCP powder magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 1 week*

Fig 25: *SEM image of cement prepared with no activated TCP powder with DOPA magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 12 hours*

Fig 26: *SEM image of cement prepared with no activated TCP powder with DOPA by 2Kx zoom on left and 10kx zoom on right set for hardenning for 1 week*

Fig 27: *SEM image of cement prepared with no activated TCP powder with DOPA+ Iodine magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 12 hours*

Fig 28: *SEM image of cement prepared with no activated TCP powder with DOPA+ Iodine magnified by 2Kx zoom on left and 10kx zoom on right set for hardenning for 1 week*