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Calcium Phosphate Materials in Bone Tissue Engineering

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SUMMARY

Calcium phosphates, together with polymers, are most commonly used materials in bone engineering since their composition is similar to bone. They are used to fulfill various defects caused by injury or bone disease, as well as for the preparation of endodontic mixtures. Because of their great importance in dentistry, these materials are given special attention in the current paper. This paper is a part of the monograph entitled "Nanomedicine, the Greatest Challenge of the 21st Century", which attracted great interest of technical and professional communities in different areas of medicine. Also for the last two years this book is promoted by the Student Cultural Centre as the only national book chosen in the narrowest election. That fact is very important for young researchers who study tissue engineering, endodontics and implantology.

Keywords: calcium phosphates; tissue engineering; endodontics; nanomedicine

INTRODUCTION

Materials used for the treatment of bone tissue should have the following characteristics: biocompatibility, good mechanical properties, porosity, and rapid degradation, as well as the possibility of easy sterilization, without changing its chemical structure and bioactivity [1, 2].

Bio-ceramic materials are considered to be biocompatible, hard, with relatively low resistance to stress, excellent compressive strength, high resistance to abrasion and suitable low friction characteristics which make them suitable for tissue engineering of hard tissues. From the point of view of their function in living systems, bioactive ceramics are divided into bio-inert ceramics, porous ceramics, bioactive ceramic materials (including bioglass, hydroxyapatite and bioactive glass ceramic) and resorptive ceramic materials. After placing a bioceramic implant four types of body response can be expected: necrosis of surrounding tissue, formation of fibrous tissue, bond at the interface between the tissue and implant and gradual replacement of surrounding tissue with new tissue [1, 3, 4, 5]. Important bioceramic materials include calcium orthophosphate systems and systems based on different calcium silicate minerals.

CALCIUM ORTHOPHOSPHATES

Calcium orthophosphates are comprised of calcium (Ca^{2+}), phosphorus (P^{5+}) and oxygen (O^{2-}). Chemical composition of many calcium orthophosphates includes

hydrogen. Different combinations of CaO and P_2O_5 (in the presence of water or without its presence) allow obtaining a variety of calcium phosphates. Calcium orthophosphates have special importance as biomaterials, where orthophosphate groups net represents the basic structural element that defines layout of all other atoms. Most of orthophosphates show low solubility in water, no solubility in alkali and good solubility in acids. From biological aspect calcium orthophosphates are main components of bone tissue in mammals [1, 6, 7].

MEMBERS OF ORTHOPHOSPHATE FAMILY

All calcium orthophosphates can be grouped into three main structural types: i) apatites, including hydroxyapatite, fluorapatite, calcium deficient apatite, octacalcium phosphate and tetracalcium phosphate; ii) glasserites, named after the mineral glasserite, including polymorphs of tricalcium phosphate and amorphous calcium phosphate; and iii) compounds that contain Ca PO_4 plates, which include dicalcium phosphate dihydrate, anhydrous dicalcium phosphate, monocalcium phosphate monohydrate and anhydrous monocalcium phosphate. According to some researchers, all calcium orthophosphates belong to the group of deformed glasserites with varying degree of distortion [8, 9, 10]. Table 1 shows the most important properties of solubility of calcium orthophosphates [1, 3, 11], while Table 2 shows their structural characteristics [1, 12, 13].

Table 1. Basic solubility characteristics of calcium orthophosphates**Tabela 1.** Osnovne osobine rastvorljivosti kalcijum-ortofosfata

Ionic ratio Ca/P Jonski odnos Ca/P	Name of compound Naziv jedinjenja	Abbr. Skr.	Chemical formula Hemijska formula	Solubility at 25°C (log Ks) Rastvorljivost na 25°C (log Ks)	Solubility at 25°C (g/L) Rastvorljivost na 25°C (g/l)	pH when stable in aqueous solution at 25°C Oblast pH stabilnosti u vodenom rastvoru na 25°C
0.5	Monocalcium phosphate monohydrate Monokalcijum-fosfat monohidrat	MPCM	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	1.14	18	0.0-2.0
0.5	Anhydrous monocalcium phosphate Anhidrovani monokalcijum-fosfat	MPCA	$\text{Ca}(\text{H}_2\text{PO}_4)_2$	1.14	17	-
1.0	Dicalcium phosphate dihydrate (brushite) Dikalcijum-fosfat dihidrat (brušit)	DCDP	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	6.59	0.088	2.0-6.0
1.0	Anhydrous dicalcium phosphate (monetite) Anhidrovani dikalcijum-fosfat (monetit)	DCPA	CaHPO_4	6.90	0.048	-
1.33	Orthocalcium phosphate Ortokalcijum-fosfat	OCP	$\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$	96.6	0.0081	5.5-7.0
1.5	α -tricalcium phosphate α trikalcijum-fosfat	α -TCP	$\alpha\text{-Ca}_3\text{PO}_4$	25.5	0.0025	-
1.5	β -tricalcium phosphate β trikalcijum-fosfat	β -TCP	$\beta\text{-Ca}_3\text{PO}_4$	28.9	0.0005	-
1.2-2.2	Amorphous calcium phosphate Amorfni kalcijum-fosfat	ACP	$\text{Ca}_x\text{H}_y(\text{PO}_4)_z \cdot n\text{H}_2\text{O}$; $n=3-4.5$	-	-	5-12
1.5-1.67	Calcium deficient hydroxyapatite Kalcijum-deficijentni hidroksiapatit	CDHA	$\text{Ca}_{10-x}(\text{HPO}_4)_6(\text{OH})_{2+x}$; ($0 \leq x \leq 1$)	85.1	0.0094	6.5-9.5
1.67	Hydroxyapatite Hidroksiapatit	HA	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	116.8	0,0003	9.5-12
1.67	Fluoroapatite Fluorapatit	FA	$\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$	120.0	0.0002	7-12
2.0	Tetracalcium phosphate Tetrakalcijum-fosfat	TTCP	$\text{Ca}_4(\text{PO}_4)_2\text{O}$	38-44	0.0007	-

Table 2. Crystallographic data of calcium orthophosphates**Tabela 2.** Kristalografski podaci kalcijum-ortofosfata

Compound Jedinjenje	Spacial group Prostorna grupa	Lattice parameters (Å or °) Parametri rešetke (Å ili °)		Z	Density (g/cm ³) Gustina (g/cm ³)
MCPM	Triclinic Triklinična P $\bar{1}$	a=5.6261(5) b=11.889(2) c=6.4731(8)	α =98.633(6) β =118.262(6) γ =83.344(6)	2	2.23
MCPA	Triclinic Triklinična P $\bar{1}$	a=7.5577(5) b=8.2531(6) c=5.5504(3)	α =109.87(1) β =93.68(1) γ =109.15(1)	2	2.58
DCPD	Monoclinic Monoklinična la	a=5.812(2) b=15.180(3) c=6.239(2)	β =116.42(3)	4	2.32
DCPA	Triclinic Triklinična P $\bar{1}$	a=6.910(1) b=6.627(2) c=6.998(2)	α =96.34(2) β =103.82(2) γ =88.33(2)	4	2.89
OCP	Triclinic Triklinična P $\bar{1}$	a=19.692(4) b=9.523(2) c=6.835(2)	α =90.15(2) β =92.54(2) γ =108.65(1)	1	2.61
α -TCP	Monoclinic Monoklinična P $2_1/a$	a=12.887(2) b=27.280(4) c=15.219(2)	β =126.20(1)	24	2.86
β -TCP	Rombohedral Romboedralna R3Ch	a=b=10.4183(5) c=37.3464(23)	γ =120	21	3.08
HA	Monoclinic or hexagonal Monoklinična ili heksagonalna P $2_1/b$; P $6_3/m$	a=9.84214(8) b=2a c=6.8814(7) γ =120 (monoclinic/ monoklinična)	a=b=9.4302(5) c=6.8911(2) γ =120 (hexagonal/ heksagonalna)	4 monoclinic, 2 hexagonal 4 monoklinična, 2 heksagonalna	3.16
FA	Hexagonal Heksagonalna P $6_3/m$	a=b=9.367 c=6.884	γ =120	2	3.20
TTCP	Monoclinic Monoklinična P $2_1/a$	a=7.023(1) b=11.986(4) c=9.473(2)	β =90.90(1)	4	3.05

Monocalcium phosphate monohydrate

Monocalcium phosphate monohydrate (MCPM), or calcium dihydrogen phosphate monohydrate is an acidic water-soluble orthophosphate. It precipitates under conditions of high acidity, while at 100°C it releases the molecule of water and becomes anhydrous monocalcium phosphate (AMCP). It is not present in biological calcification because of its high acidity. It is also not biocompatible, but still used in medicine as a component of self-setting orthophosphate cements [1, 14].

Anhydrous monocalcium phosphate

Anhydrous monocalcium phosphate, AMCP, or anhydrous calcium dihydrogen phosphate, crystallizes under the same conditions as MCPM at temperatures above 100°C. It does not have application in medicine and has very hygroscopic properties [1, 6, 14].

Dicalcium phosphate dihydrate

Dicalcium phosphate dihydrate or calcium hydrogen phosphate dihydrate, DCDP, also called brushite, readily crystallize from aqueous solutions at $\text{pH} \leq 6.5$. It is transformed into anhydrous dicalcium phosphate above 80°C. CaPO_4 consists of chains which are mounted parallel and connected to each other through the layers of water molecules. Using X-ray diffraction the atomic structure of the (010) interfacial DCDP was determined wherein water molecules can be found (Figure 1). DCDP is biologically very important mineral because it is present in pathological calcification (dental calculus (mineral and organic deposits on teeth)), crystalluria (crystals found in urine), hondro-

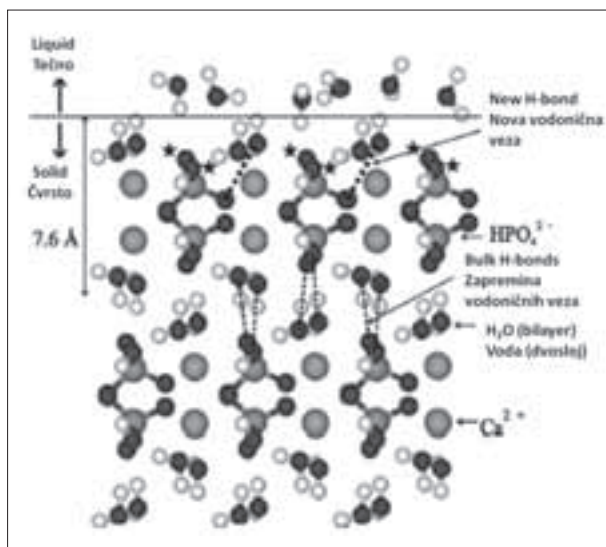


Figure 1. Schematic representation of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$; Water is incorporated into the bilayer. Some of the hydrogen bonds in bulk were presented with dotted lines.

Slika 1. Shematski prikaz $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$; voda je ugrađena u dvo-sloju. Neke vodonične veze u zapremini predstavljene su tačkastim linijama.

calcinosis (symptoms of rheumatic diseases caused by accumulation of dicalcium phosphate in joints), urolithiasis (stones in urine), as well as in dental caries. It is used as a component of calcium phosphate cements in medicine as well as an intermediate in teeth remineralisation. Brushite or DCDP, is also a model system for studying the process of biomineralization. It grows slowly from solution in the form of plates, showing interesting morphology [1, 15, 16, 17].

Anhydrous dicalcium phosphate

Anhydrous dicalcium phosphate or anhydrous calcium hydrogen phosphate, DCPA, also known as mineral monetite is the anhydrous form of dicalcium phosphate dihydrate. It is less soluble in water due to the absence of water inclusion in its structure. It crystallizes from aqueous solution at 100°C. Most commonly it is sintered at 300°C. It is present neither in normal nor pathological calcification. It is used as part of calcium phosphate cements in medicine [1, 18].

Octacalcium phosphate

Octacalcium phosphate, or octacalcium bis (hydrogen phosphate) tetrakis (phosphate) pentahydrate, OCP, is unstable transient intermediate which is generated during precipitation in aqueous solution from thermodynamically more stable calcium-deficient hydroxyapatite. OCP has great biological significance since it represents stable component of human dental and urinary calculi. It first precipitates as intermediate phase in the formation of enamel and bone through gradual hydrolysis of OCP. It plays an important role *in vivo* in the formation of apatite biominerals. Although not found in vascular calcification, as it is highly emphasized in numerous studies, it is probably OCP the precursor of biological apatite found on natural and prosthetic heart valves. It is also used in surgery for the implantation of bone defects. Stability of OCP is another reason for its importance, only hydroxyapatite shows better stability [1, 19, 20, 21].

Beta-tricalcium phosphate

Beta-tricalcium phosphate or β -tribasic calcium phosphate, β -TCP, cannot precipitate from the aqueous solution. It is a high temperature phase obtained at temperatures above 800°C by thermal decomposition of calcium deficient hydroxyapatite or by reaction in the solid phase of acidic calcium orthophosphate (anhydrous dicalcium phosphate) with CaO (Figure 2). It can be produced by bone calcinations as so-called "Bone ash". It has never been found in biological calcifications. As Mg- substituted form of tricalcium phosphate it is present in the form of mineral whitlockite (β -tricalcium phosphate magnesium, β -TCMP) in dental calculus, urinary calculus, dental caries, salivary stones, arthritis cartilage and as a deposit in soft tissue. It has not been observed in the composition of dentin and enamel. It is used in biomedical applications as bone cement. In

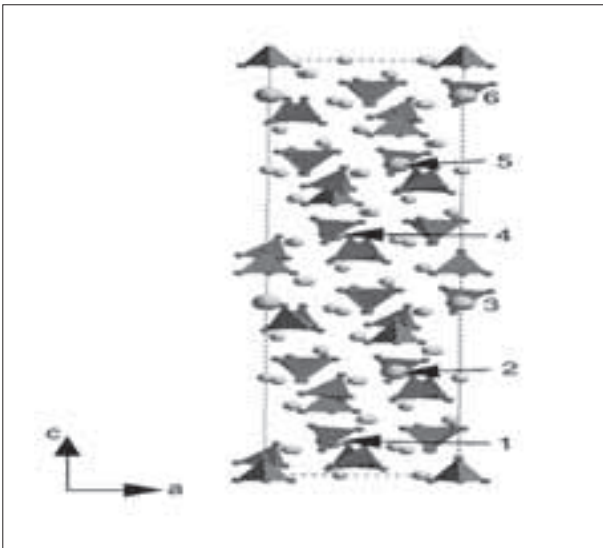


Figure 2. Beta-tricalcium phosphate: unit cell projected on (a, c) plane; tetrahedron represents PO_4 groups and small balls represent Ca atoms while large balls are partially occupied Ca.

Slika 2. Beta trikalcijum-fosfat: jedinična ćelija projektovana na (a, c) ravan; tetraedar predstavlja PO_4 grupe, a male loptice atome Ca, dok su velike loptice mesta Ca koja su polovično zaposednuta.

combination with hydroxyapatite it forms biphasic calcium phosphate, BCP. β -TCP and two phase calcium phosphate are widely used as bone substitutes [6, 22].

The use of β -calcium phosphate in bone tissue engineering

Bone defects present powerful medical and socioeconomic challenge. Although autologous bone grafts are considered gold standard for reconstruction of bone defects there still some drawbacks of this treatment, such as hypersensitivity of donor and limited size of graft. Designing bio-artificial bone tissue has helped to overcome these problems. Various types of biomaterials can be used in reconstructions. They should meet a number of criteria, such as biocompatibility, ie. non-immunogenicity and non-toxicity, absorptivity, osteoconductivity, easy preparation and sterilization and good mechanical properties. In addition, porosity of biomaterial is very important for scaffold preparation, because it plays an important role *in vitro* from the viewpoint of mechanical properties or *in vivo*, from the viewpoint of bone resorption and new bone growth. Pore interconnection is particularly important for integration of new bone in material because scaffold should provide skeletal support for osteogenic cells growth in early stages and create enough space for the formation of new bone [1, 23, 24, 25].

The next step in the development of porous structure is the choice of available resources of cells which allow their isolation and expansion in larger numbers. Ideal source of cells should be readily proliferative, non-immunogenic, and to have similar expression of proteins that regenerate tissues. It is known that bone marrow stem cells (BMSC) provide good source of osteogenic cells for the formation of new bone but also secrete growth factors that recruit

natural cells to migrate into the defects. Under appropriate conditions *in vitro* BMSC can differentiate into bone, cartilage, adipose tissue and hematopoietic- supporting stroma cells. Osteogenic potential of BMSC *in vivo* is particularly evident when they are found on the ceramic scaffold that accelerates bone formation. From the standpoint of bone tissue engineering BMSC have numerous advantages. Their isolation is quick, they easily adhere, have high proliferative potential (as rule BMSC have osteogenic transformation), bone formation is not correlated with cell changes if cells maintain their proliferative potential, and freezing conditions do not affect BMSC osteogenic potential and make storage easier [1, 25, 26, 27].

Some studies have shown bone inductance after implantation of calcium phosphate ceramics in extra skeletal places, such as muscles and subcutaneous implantation in dogs, baboons, sheep and pigs. After implantation of porous β -TCP with or without BMSC in rat muscles, bone formation, as shown by some studies was analyzed histo-morphometrically after 1, 4 and 8 weeks in each of the above groups of animals. It was found that newly formed bone per unit area of β -TCP grows per unit of time, resulting in continual resorption of β -TCP and bone formation after four weeks with marked increase in bone mass after eight weeks. Another important factor is cell-based construct which consists of biodegradable scaffold β -TCP and osteogenically induced BMSC stem cells. After eight weeks osteoblasts of cubic forms were found to form new bone, suggesting that these cells are active, while the scaffold alone was not sufficient to form new bone. The combination of scaffold with β -TCP and BMSC provide better local conditions for osseointegration. Such construct, as shown in some studies, is the critical factor for bone induction which has great significance for potential use of combined materials in orthopedic reconstructive procedures [1, 25, 26, 27].

Alpha-tricalcium phosphate

Alpha-tricalcium phosphate or α -tribasic calcium phosphate, α -TCP, is obtained by heat treatment of β -TCP at about 1125°C why it may be considered as its high-tem-

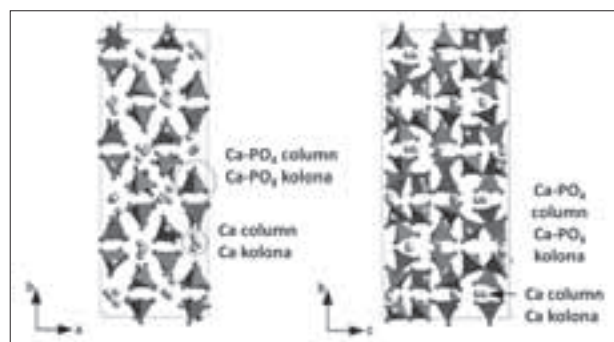


Figure 3. Alpha-tricalcium phosphate cells, left: projected on (a, b) plane, right: projected on (b, c) plane. Tetrahedron represents PO_4 group, bright balls represent Ca atoms.

Slika 3. Alfa trikalcijum-fosfatna ćelija: levo – projektovana na (a, b) ravan, desno – projektovana na (b, c) ravan. Tetraedar predstavlja PO_4 grupe, a svetle lopte predstavljaju atome Ca.

perature phase. It can also be produced at temperatures between 800 and 1000°C, when it is stabilized with silicon (silicon stabilized α -tricalcium phosphate). α - and β -tricalcium phosphate, although the same chemical composition, they differ in their structure and solubility (β -TCP is more stable than α -TCP). α -TCP is much more reactive in aqueous solutions than β -TCP and has higher specific energy and readily hydrolyze to the mixture of the other calcium phosphates (Figure 3). It has never been found in biological calcifications. In medicine it is used in different cements. Silicon stabilized α -TCP (as a two-phase composite with hydroxyapatite) is used for designing porous ceramic scaffolds that can be used as artificial bone substitutes [1, 28, 29].

Amorphous calcium phosphate

Amorphous calcium phosphate, ACP, presents intermediate phase during calcium orthophosphate formation in aqueous systems. ACP usually precipitates from solution after rapid mixing of the solution that contains calcium and orthophosphate ions. It is formed at the beginning of precipitation because of its lower surface energy than surface energy of OCP and hydroxyapatite. Amorphous proportion within ACP increases with increase of Ca^{2+} and PO_4^{3-} concentration especially at higher pH and lower temperatures crystallization. At increased temperature, by moderate mixing of stock solution of ACP a slow re-crystallization and formation of better crystallized calcium-deficient hydroxyapatite is performed. The time of ACP is stability in an aqueous solution depends on the presence of molecules, and additive ions, pH, ionic strength and temperature [1, 30].

Biological ACP (often with impurities of ions Na, Mg, and pyrophosphate carbonate) has been found in pathological calcifications of soft tissues (heart valve calcification in uremia, renal disease). In medicine, ACP is used in calcium orthophosphate cements and as filler in dentistry. His composites with bioactive polymers are also used in dentistry and surgery. It is also used as inert filler for medications.

Amorphous calcium phosphate is in most cases the solid phase at $\text{pH} \geq 7$, and when in sufficiently high concentration it precipitates immediately. It is kinetically driven process. Rapid mixing of highly concentrated solutions causes strong interaction between ions and rapid coalescence of irregular, highly hydrated clusters, large enough to be secreted from the solution in gel-like structures before they relocate in nuclei which then grow as crystals. This structural arrangement is unstable. Moreover, in this process ACP is disappearing because it transforms in more stable crystalline phases such as OCP and calcium deficient hydroxyapatite.

While the expansion of crystalline domains consumes surrounding calcium and orthophosphate ions (or their pairs and clusters) and release hydrated proton, mechanical strength decreases. Finally, under the influence of fluid shear stress, primary particles collapse and release crystallites causing rapid precipitation of calcium orthophos-

phates, together with previously captured hydrated protons of primary particles which causes drop in pH. It has been clearly demonstrated in many practical examples that crystallization in number of places within amorphous particles eventually leads to rapid precipitation of calcium phosphate from oversaturated solution [1, 31-34].

CONCLUSION

Calcium phosphate systems together with materials based on active calcium silicate systems are most commonly used materials in the engineering of bone tissue. Since there are large number of these materials including their various combinations, knowledge about their basic characteristics is essential for their use in various indications, especially in the treatment of bone defects. These biomaterials have excellent biocompatibility, absorptivity, osteoconductivity, porosity and good mechanical properties. Porosity of these materials is particularly important for the development of scaffolds and integration of new bone in material as skeletal support for the growth of osteogenic cells and formation of new bone.

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Kalcijumfosfatni materijali u inženjerstvu koštanog tkiva

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KRATAK SADRŽAJ

Kalcijum-fosfati, zajedno s nekim polimerima, najviše su korišćeni materijali u inženjerstvu koštanog tkiva, budući da su po sastavu bliski prirodnoj kosti. Koriste se za ispunu različitih oštećenja nastalih usled povreda ili bolesti koštanog tkiva, kao i za pripremu endodontskih mešavina za primenu u stomatologiji. Zbog izuzetnog značaja u stomatologiji, kalcijumfosfatni materijali zaslužuju posebno mesto, pa će im u okviru ovog rada, ali i radova koji će uslediti, biti posvećena posebna pažnja. Radovi su najvećim delom sastavni deo monografije pod nazivom „Nanomedicina, najveći izazov 21. veka“, koja je pobudila veliko interesovanje stručne i profesionalne javnosti usmerene ka različitim oblastima medicine i koju je već dve godine zaredom Studentski kulturni centar, kao jedinu knjigu domaćeg autora, promovisao kao knjigu najužeg izbora. Verujemo da je ta činjenica posebno važna za mlađe istraživače koji se bave problemima inženjerstva tkiva, endodoncijom i implantologijom.

Ključne reči: kalcijum-fosfati; inženjerstvo tkiva; endodoncija; nanomedicina

UVOD

Neophodne osobine materijala koji bi imali primenu u terapiji koštanog tkiva uključuju biokompatibilnost, dobra mehanička svojstva, poroznost i brzu degradaciju materijala, kao i mogućnost lake sterilizacije materijala, pri čemu ne dolazi do značajnih promena u njegovom hemijskom sastavu i bioaktivnosti [1, 2].

Biokeramički materijali se smatraju biokompatibilnim, tvrdim, s relativno slabom otpornošću na naprezanje, odličnom pritiskom čvrstoćom, visokom otpornošću na habanje i pogodnim niskim frikcionim osobinama, te su kao takvi najpogodniji za tkivno inženjerstvo tvrdih tkiva. U pogledu načina njihovog funkcionisanja u živim sistemima, biokeramički materijali se dele na: bioinertne, porozne, bioaktivne (koji obuhvataju biostaklo, bioaktivnu staklokeramiku i hidroksiapatit) i bioresorptivne keramičke materijale. Nakon ugrađivanja biokeramičkog implanta, kao odgovor organizma mogu se javiti četiri osnovna tipa reakcija: odumiranje okolnog tkiva, formiranje vlaknastog tkiva, obrazovanje veze na dodirnoj površini između prirodnog tkiva i implanta i postepena zamena okolnog tkiva novim [1, 3, 4, 5]. Među biokeramičkim materijalima posebno mesto pripada kalcijumortofosfatnim i sistemima zasnovanim na primeni različitih kalcijumsilikatnih minerala.

KALCIJUM-ORTOFOSFATI

Kalcijum-ortofosfati se sastoje od kalcijuma (Ca^{2+}), fosfora (P^{5+}) i kiseonika (O^{2-}). Hemijski sastav mnogih kalcijum-ortofosfata uključuje i vodonik. Različite kombinacije CaO i P_2O_5 (u prisustvu vode ili bez nje) omogućavaju dobijanje veoma raznovrsnih kalcijumfosfatnih jedinjenja. Poseban značaj kao biomaterijali imaju samo kalcijum-ortofosfati, kod kojih je mreža ortofosfatnih grupa osnovni strukturni element koji određuje raspored svih ostalih atoma koji se nalaze u sastavu ortofosfata. Većina ortofosfata je slabo rastvorljiva u vodi, nerastvorljiva u alkalinama i dobro rastvorljiva u kiselinama. Biološki posmatrano, ortofosfati kalcijuma su glavna komponenta svih koštanih tkiva sisara [1, 6, 7].

ČLANOVI ORTOFOSFATNE PORODICE

Svi kalcijum-ortofosfati mogu se grupisati u tri osnovna strukturna tipa: I) apatiti, koji uključuju hidroksiapatit, fluorapatit, kalcijum-deficijentni apatit, oktakalcijum-fosfat i tetrakalcijum-fosfat; II) glaseriti, imenovani po mineralu glaseritu, koji uključuju polimorfe trikalcijum-fosfata i možda amorfni kalcijum-fosfat; i III) jedinjenja koja sadrže Ca-PO_4 ploče, koja uključuju dikalcijum-fosfat dihidrat, anhidrovani dikalcijum-fosfat, monokalcijum-fosfat monohidrat i anhidrid monokalcijum-fosfata. Prema nekim istraživačima, svi ortofosfati kalcijuma pripadaju deformisanim glaseritnim tipovima strukture, s različitim stepenom distorzije [8, 9, 10]. Tabela 1 prikazuje najvažnije osobine rastvorljivosti kalcijum-ortofosfata [1, 3, 11], dok tabela 2 pokazuje njihove strukturne osobenosti [1, 12, 13].

Monokalcijum-fosfat monohidrat

Monokalcijum-fosfat monohidrat (MCPM), ili kalcijum-dihidrogenfosfat monohidrat, kiseli je ortofosfat rastvorljiv u vodi. Precipitira u uslovima visoke kiselosti, dok na 100°C otpušta molekul vode i prelazi u anhidrovani monokalcijum-fosfat (MCPA). Ne nalazi se u biološkim kalcifikacijama zbog svoje visoke kiselosti. Nije biokompatibilan, ali se ipak koristi u medicini kao komponenta samoočvršćavajućih ortofosfatnih vrsta cementa [1, 14].

Anhidrovani monokalcijum-fosfat

Anhidrovani monokalcijum-fosfat (MCPA), ili anhidrovani kalcijum-dihidrogen fosfat, kristališe pod istim uslovima kao i MCPM na temperaturama većim od 100°C . Ne primenjuju se u medicini. Ima izrazito higroskopne osobine [1, 6, 14].

Dikalcijum-fosfat dihidrat

Dikalcijum-fosfat dihidrat (DCDP), ili kalcijum-hidrogenfosfat dihidrat, poznat i pod nazivom brušit, lako kristališe iz vodenih

rastvora pri vrednosti $\text{pH} \leq 6,5$. Transformiše se u anhidrovani dikalcijum-fosfat na temperaturama većim od 80°C . Sastoji se od lanaca CaPO_4 koji su postavljeni paralelno jedan u odnosu na drugi, povezujući se međusobno slojevito preko molekula vode. Pomoću difrakcije rendgenskih zraka utvrđena je atomska struktura (010) međupovršine DCDP, u kojoj se nalaze molekuli vode (Slika 1). DCDP je biološki vrlo značajan mineral jer se nalazi u patološkim kalcifikacijama (zubnim kalkulusima – mineralnim i organskim depozitima na zubima), kristalurijama (kristali pronađeni u mokraći), hondrokalcinozama (simptomi reumatske bolesti na koje ukazuje akumulacija dikalcijum-fosfata u vezivnim tkivima zglobova), urolitijazi (kamenje u mokraći) i karijesnim lezijama. U medicini se koristi kao komponenta kalcijum-fosfatnih vrsta cementa i kao intermedijer kod remineralizacije zuba. Brušit je takođe model sistem za proučavanje procesa biomineralizacije. Raste sporo iz rastvora u formi ploča, koje pokazuju zanimljivu morfologiju [1, 15, 16, 17].

Anhidrovani dikalcijum-fosfat

Anhidrovani dikalcijum-fosfat (DCPA), ili anhidrovani kalcijum-hidrogen fosfat, poznat kao mineral monetit, anhidrovani je oblik dikalcijum-fosfata dihidrata. Manje je rastvorljiv u vodi od dihidrata zbog izostanka inkluzija vode u svojoj rešetki. Kristališe iz vodenog rastvora na 100°C . Najčešće se sinteruje na 300°C . Ne nalazi se ni u normalnim, ni u patološkim kalcifikacijama. Koristi se u kalcijumfosfatnim vrstama cementa u medicini [1, 18].

Oktakalcijum-fosfat

Oktakalcijum-fosfat (OCP), ili oktakalcijum-bis(hidrogen fosfat) tetrakis(fosfat) pentahidrat, nestabilni je prelazni intermedijer koji nastaje tokom precipitacije u vodenom rastvoru termodinamički znatno stabilnijeg kalcijum-deficijentnog hidroksiapatita. OCP ima veoma veliki biološki značaj, jer predstavlja stabilnu komponentu ljudskih zubnih i mokraćnih kalkulusa. On prvo precipitira kao polazna faza pri nastajanju minerala enamela i kao polazna faza u obrazovanju kosti kroz postepenu hidrolizu OCP. Igra veoma značajnu ulogu *in vivo* u stvaranju apatitnih biominerala. Mada nije pronađen u vaskularnoj kalcifikaciji, u brojnim istraživanjima je naglašeno da je najverovatnije upravo OCP osnovna prekursorska faza nastajanja biološkog apatita nađenog u prirodnim i protetičkim srčananim zaliscima. U hirurgiji se koristi za implantaciju koštanih defekata. Stabilnost OCP je drugi razlog njegovog značaja, jer se, prema stepenu stabilnosti u brojnim fiziološkim medijima, OCP nalazi odmah iza hidroksiapatita [1, 19, 20, 21].

Beta trikalcijum-fosfat

Beta trikalcijum-fosfat (β -TCP), ili β -tribazni kalcijum-fosfat, ne može da precipitira iz vodenog rastvora. To je tzv. faza visoke temperature, koja se dobija na temperaturama iznad 800°C , termičkim razlaganjem kalcijum-deficijentnog hidroksiapatita ili reakcijom u čvrstoj fazi kiselog kalcijum-ortofosfata (anhidrovani dikalcijum-fosfata) sa CaO (Slika 2). Dobija se i kal-

cinacijom kosti, kao tzv. koštani pepeo. Nikad nije pronađen u biološkim kalcifikacijama. Kao Mg-supstituisani oblik trikalcijum-fosfata nalazi je u obliku minerala vajtlokita (β -trikalcijum magnezijum-fosfata – β -TCMP) u zubnim kalkulusima, mokraćnom kamencu, karijesu, pljuvačnim kamencima, artritisnoj hrskavici i kao depozit u mekim tkivima. Nije uočen u sastavu dentina i gleđi. Koristi se u biomedicini kao koštani cement. U kombinaciji s hidroksiapatitom stvara dvofazni kalcijum-fosfat (BCP). β -TCP i BCP široko se koriste u zameni kosti [6, 22].

Primena beta kalcijum-fosfata u inženjerstvu koštanih tkiva

Oštećenja kosti su veliki medicinski i socioekonomski izazov. Mada se autologni koštani graftovi smatraju zlatnim standardom za rekonstrukciju koštanih oštećenja, i kod njih se uočavaju brojni nedostaci, kao što su preostljivost davaoca i ograničenje veličine grafta. Dizajniranje bioveštačkih koštanih tkiva pomaže da se prevaziđe takav problem. Razne vrste biomaterijala se koriste u rekonstruktivnim indikacijama, zbog čega pobuđuju sve veće interesovanje. Takvi materijali treba da zadovolje razne kriterijume, kao što su: biokompatibilnost, tj. neimunogenost i netoksičnost, apsorptivnost, osteokonduktivnost, lakoća izrade i sterilizacije i dobre mehaničke osobine. Uz to veoma je bitna i poroznost biomaterijala za izradu skafolda, jer ona igra važnu ulogu u uslovima *in vitro* sa stanovišta mehaničkih osobina materijala, odnosno *in vivo* sa stanovišta procesa resorpcije i rasta nove kosti. Međupovezanost pora je posebno važna za integraciju nove kosti u materijal, jer skafold treba da obezbedi podršku skeleta za rast osteogenih ćelija u ranoj fazi i stvori dovoljno prostora za obrazovanje nove kosti [1, 23, 24, 25].

Sledeći korak u razvoju prikladne porozne strukture jeste izbor raspoloživog izvora ćelija koji dopušta njihovu izolaciju i ekspanziju u većem broju. Idealan ćelijski izvor treba da je lako proliferativan, da je neimunogen i da ima ekspresiju proteina sličnih tkivima koja se regenerišu. Poznato je da zasejane matične ćelije iz koštane srži (engl. *bone marrow mesenchymal stem cells* – BMSCs) ne obezbeđuju samo izvor osteogenih ćelija kao izvor za obrazovanje nove kosti, nego i luče faktore rasta da bi regrutovali prirodne ćelije da migriraju u oštećena mesta. Pod odgovarajućim uslovima *in vitro* BMSCs se diferenciraju u kost, hrskavicu, masna tkiva i hematopoetske potporne stroma ćelije. U uslovima *in vivo* osteogeni potencijal BMSCs posebno dolazi do izražaja kad se one nalaze na keramičkim skafoldima koji ubrzavaju obrazovanje kosti. Sa stanovišta inženjerstva koštanih tkiva, BMSCs imaju brojne prednosti. Njihova izolacija je laka i pokazuju primarnu sposobnost da lako adheriraju, imaju visok proliferativni potencijal (po pravilu, BMSCs odlikuje osteogeni put transformacije), obrazovanje kosti nije u korelaciji sa brojem ćelijskih promena dok god proliferativne ćelije održavaju svoj proliferativni potencijal, a uslovi zamrzavanja ne utiču na osteogeni potencijal BMSCs, već samo olakšavaju njihovo skladištenje [1, 25, 26, 27].

Neka istraživanja su pokazala koštanu induktivnost posle implantacije kalcijumfosfatne keramike u vankoštanu mesta, kao što su mišići, i potkožne implantacije kod pasa, babuna, ovaca i svinja. Posle implantacije poroznog β -TCP sa BMSCs ili bez njih u mišiću pacova, obrazovanje kosti, kao što pokazuju neke studije, analizirano je histiomorfometrijski posle nedelju

dana, četiri nedelje i osam nedelja u svakoj navedenoj grupi životinja. Utvrđeno je da novoformirana kost po jedinici površine β -TCP raste u jedinici vremena, dovodeći do neprestane resorpcije β -TCP i obrazovanja nove kosti posle četiri nedelje s izrazitim povećanjem koštane mase nakon osam nedelja. Drugi važan faktor je konstrukt zasnovan na ćelijama, koji se sastoji od biodegradibilnog skafolda β -TCP i osteogenetski indukovanih BMSCs. Posle osam nedelja pronađeni su osteoblasti kubičnog oblika, koji su obrazovali novu kost, ukazujući na to da su ove ćelije aktivne, dok sam skafold nije bio dovoljan da bi nastala nova kost. Kombinacija skafolda sa β -TCP i BMSCs obezbeđuje bolje lokalne uslove za osteointegraciju. Takav konstrukt, kao što je pokazano u nekim istraživanjima, kritičan je faktor za indukciju kosti, što je veoma značajno za potencijalnu primenu tako kombinovanih materijala u ortopedskim rekonstruktivnim zahvatima [1, 25, 26, 27].

Alfa trikalcijum-fosfat

Alfa trikalcijum-fosfat (α -TCP), ili α -tribazni kalcijum-fosfat, dobija se najčešće termičkim tretmanom β -TCP na oko 1125°C, zbog čega se može smatrati i njegovom visokotemperaturnom fazom. Takođe, moguće ga je dobiti i na temperaturama i između 800°C i 1000°C, kad je stabilisan sa silicijumom (silicijumom stabilisani α -TCP). Alfa i beta trikalcijum-fosfat, mada imaju isti hemijski sastav, razlikuju se po svojoj strukturi i rastvorljivosti (β -TCP je stabilniji od α -TCP). α -TCP je znatno reaktivniji u vodenim rastvorima od β -TCP, ima veću specifičnu energiju i lako hidrolizuje u smesu drugih kalcijum-fosfata (Slika 3). Nikad nije primećen u biološkim kalcifikacijama. U medicini se koristi u odgovarajućim cementima. Silicijumom stabilisani α -TCP (kao dvofazni kompozit sa hidroksiapatitom) koristi se kod dizajniranja poroznih keramičkih skafolda koje je moguće koristiti kao veštačke zamenike kosti [1, 28, 29].

Amorfni kalcijum-fosfat

Amorfni kalcijum-fosfat (ACP) javlja se kao prelazna faza tokom formiranja kalcijumortofosfata u vodenim sistemima. ACP najčešće prvo precipitira iz presičenog rastvora pri brzom mešanju rastvora koji sadrži jone kalcijuma i ortofosfatne jone. On se formira na početku precipitacije zbog toga što je njegova površinska energija manja od površinske energije OCP i hidroksiapatita. Amorfni udeo unutar ACP povećava se sa rastom koncentracije Ca^{2+} i PO_4^{3-} , posebno pri većim vrednostima pH i nižim temperaturama kristalizacije. Na povećanoj temperaturi, umerenim mešanjem matičnog rastvora ACP dolazi do

njegove spore rekristalizacije i stvaranja bolje iskristalisanog kalcijum-deficijentnog hidroksiapatita. Vreme stabilnosti ACP u vodenom rastvoru zavisi od prisustva molekula i jona aditiva, vrednosti pH, jonske jačine i temperature [1, 30].

Biološki ACP (često sa nečistoćama koje čine joni Na, Mg, karbonata i pirofosfata) pronađen je u patološkim kalcifikacijama mekih tkiva (kalcifikacija srčanih zalizaka kod uremije, bolesti bubrega). U medicini se ACP koristi u kalcijumortofosfatnim vrstama cementa i kao punilo u stomatologiji. Njegovi kompoziti sa bioaktivnim polimerima koriste se takođe u stomatologiji i hirurgiji. Koristi se i kao inertno punilo za lekove.

ACP je u većini slučajeva čvrsta faza koja pri vrednostima $\text{pH} \geq 7$ i pri dovoljno visokoj koncentraciji precipitira trenutno. To je kinetički vođen proces. Brzo mešanje visokokonzentrovanih rastvora uslovljava snažne stohastičke interakcije između jona i brzu koalescenciju u nepravilno koordinisane, visoko hidratne klastere, dovoljno velike da se izluče iz rastvora u strukturu sličnu gelu pre nego što se prerasporede u nukleuse koji potom rastu u kristale. Ovaj strukturni raspored je nestabilan. Uz to, u tom procesu nestaje ACP, jer se transformiše u stabilnije kristalne faze, kao što su OCP i kalcijum-deficijentni hidroksiapatit.

Dok se pri ekspanziji kristalnih domena troše okolni kalcijumovi i ortofosfatni joni (ili njihovi parovi i klasteri) i otpušta hidratni proton, mehanička čvrstoća slabi u međudomenskom prostoru. Na kraju, pod dejstvom napona smicanja fluida, primarne čestice popuštaju i oslobađaju kristalite uslovljavajući brzu precipitaciju kalcijum-ortofosfata, zajedno s prethodno zarobljenim hidratnim protonima primarnih čestica, što uslovljava snažan pad vrednosti pH. U mnogim praktičnim primerima nedvosmisleno je pokazano da kristalizacija u brojnim mestima unutar amorfne čestice na kraju vodi brzom precipitaciji kalcijum-fosfata iz presičenog rastvora [1, 31-34].

ZAKLJUČAK

Kalcijumfosfatni sistemi su zajedno s materijalima na bazi aktivnih kalcijumsilikatnih sistema najviše i najčešće korišćeni materijali u inženjerstvu koštanog tkiva. Budući da je reč o velikom broju ovih materijala, ali i njihovim brojnim kombinacijama, poznavanje njihovih osnovnih osobina je jedan od glavnih preduslova za njihovu primenu u različitim indikacijama, a naročito u lečenju oštećenja kosti. Ovi biomaterijali imaju izvanrednu biokompatibilnost, apsorptivnost, osteoinduktivnost, poroznost i dobre mehaničke osobine. Poroznost ovih materijala je naročito važna za izradu skafolda i integraciju nove kosti u materijal, kao skeletna podrška za rast osteogenih ćelija i obrazovanje nove kosti.