

# Mg and Si substituted hydroxyapatite: behaviour in simulated body fluid

Božana Petrović<sup>1</sup>, Maja Krstić<sup>1</sup>, Tihana Mudrinić<sup>2</sup>, Maria Čebela<sup>1</sup>, Maja Dutour Sikirić<sup>3</sup>

<sup>1</sup>Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia;

<sup>2</sup>Institute of Chemistry, Technology and Metallurgy – National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia;

<sup>3</sup>Rudjer Bošković Institute, Zagreb, Croatia

## SUMMARY

Due to its similarity with biological apatite found in vertebrate hard tissues, calcium hydroxyapatite is one of the most investigated materials in bone tissue engineering. As the biological apatite is not stoichiometric, ion substituted hydroxyapatites attract much attention since they more closely mimic the composition of natural bone. Although there are many investigations of the influence of foreign ions on the structure and physico-chemical properties of ion-substituted hydroxyapatites, there is scarce information on their behaviour in different media.

In the present study, magnesium (Mg), that plays a key role in bone metabolism and silicon (Si), that is necessary for normal skeletal development, were used as ion substitutes. The behaviour of Mg and Si substituted hydroxyapatite in simulated body fluid was investigated by XRD, FTIR and SEM. Obtained results confirmed great potential of these substituted hydroxyapatites for biomedical applications.

**Keywords:** substituted hydroxyapatite; magnesium; silicon; SBF

## INTRODUCTION

Since calcium phosphates (CP) constitute the main mineral component of the hard tissues of vertebrates, their synthetic analogues are the most commonly used materials in orthopedics and dentistry [1, 2, 3]. Specifically, the mineral phase of the bones and teeth is the basic calcium phosphate, which is being equalized with synthetic calcium hydroxyapatite (HA,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) [2]. HA exhibits excellent biocompatibility and bioactivity due to its similarity with inorganic phases of biomineralized tissues, making it the most studied CP [1–5]. It is used for numerous biomedical applications, mostly in bone tissue engineering, such as: bone scaffold, for restoration of bone and periodontal defects, as a coating on metal implants, drug delivery system and antibacterial agent.

Despite huge similarity, biological apatites differ from stoichiometric HA in several aspects, such as non-stoichiometry, small crystal sizes and poor crystallinity which are important factors associated with the relatively high solubility of biological apatites compared to stoichiometric HA [2]. Biological apatites are actually carbonated apatites containing also foreign ions, which can be incorporated in or adsorbed on the apatite crystal surface. Also, nucleation and growth of CP in biological systems occur in an environment rich in ions, which can affect the kinetics of crystallization, and further the relative stability of CP. Therefore, the study of ionic substitution in HA is significant for several reasons: a better understanding of the biomineralization processes, control of the properties of

the precipitated phase, increase of the bioactivity of the material, and delivery of the ions for treatment of the diseased bone [2, 6].

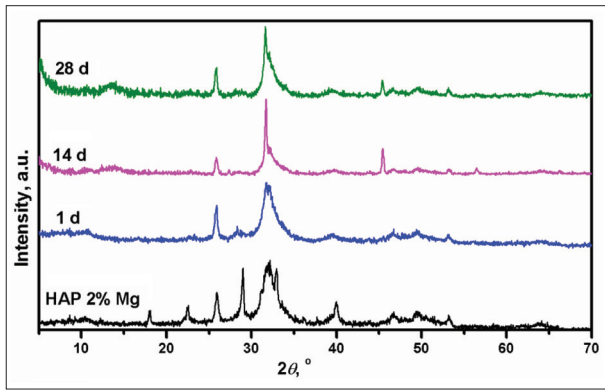
Magnesium and silicon ions are good candidates for incorporation in the HA lattice due to their biological importance. As it is known, magnesium is an important trace element in bone and teeth, and its lack causes bone fragility [2, 7]. It plays a key role in bone metabolism, since it influences osteoblast and osteoclast activity, and thereby bone growth. Silicon has a metabolic role in the bone growth and has been reported as necessary trace element for normal skeletal development, especially in the initial phases of bone formation [3, 6, 8]. Besides, simultaneous Mg/SiO<sub>4</sub> substitution was proposed as a manner to obtain material similar to biological apatite [2]. Therefore, these two elements were chosen to substitute Ca<sup>2+</sup>/PO<sub>4</sub><sup>3-</sup> in the HA lattice and investigate their behavior after immersion into simulated body fluid.

## MATERIALS AND METHODS

### Synthesis of substituted hydroxyapatite

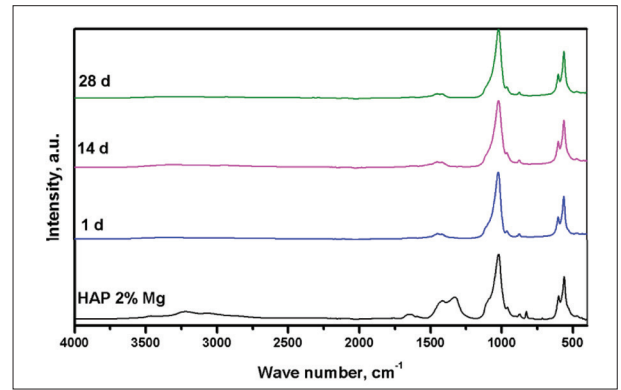
All chemicals used were purchased from Sigma Aldrich, Germany.

Mg- substituted HA was synthesized by adding (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> solution dropwise to the solution of Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O and Mg(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O. The reaction mixture was heated at 100°C under reflux for 5 hours. During



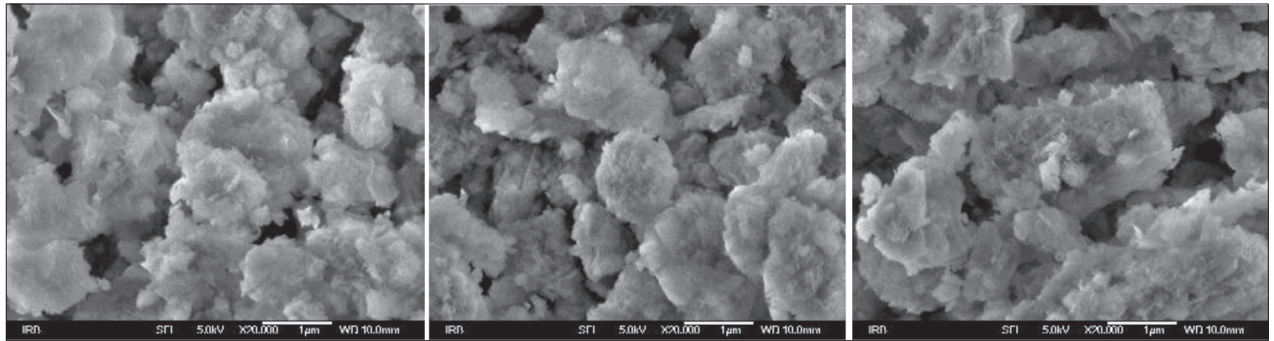
**Figure 1.** XRD patterns of HA doped with 2% Mg: the initial powder and powder obtained after immersion in corrected simulated body fluid for 1–28 days at 37°C.

**Slika 1.** XRD spektri HA dopiranog sa 2% Mg: početni prah i prahovi dobijeni posle potapanja u korigovani simulirani telesni fluid tokom 1–28 dana na 37°C.



**Figure 2.** FTIR spectra of HA doped with 2% Mg: the initial powder and powder obtained after immersion in corrected simulated body fluid for 1–28 days at 37°C

**Slika 2.** FTIR spektri HA dopiranog sa 2% Mg: početni prah i prahovi dobijeni posle potapanja u korigovani simulirani telesni fluid tokom 1–28 dana na 37°C



**Figure 3.** SEM images of HA doped with 2% Mg: a) the initial powder, b) powder after immersion in corrected simulated body fluid for 14 days, c) powder after immersion in corrected simulated body fluid for 28 days

**Slika 3.** SEM slike HA dopiranog sa 2% Mg: a) početni prah, b) prah i prahovi dobijeni nakon potapanja u korigovani simulirani telesni fluid tokom 14 dana, c) prah posle potapanja u korigovani simulirani telesni fluid tokom 28 dana

heating urea and urease were added and pH 8.8 was obtained. The product was filtered, washed with deionised water and ethanol and dried at 120°C.

Mg, Si-substituted HA was synthesized by adding solution containing  $(\text{NH}_4)_2\text{HPO}_4$  and  $\text{SiO}_2$  sol in a controlled manner to the solution of  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ . The  $\text{SiO}_2$  sol was synthesized by the procedure described by Jokanović et al. [9]. The reaction mixture was heated at 100°C, under reflux for 5 hours. During heating urea urease was added and pH 7.9 was obtained. The product was filtered, washed with deionised water and ethanol and dried at 200 °C.

### Mineralization in corrected simulated body fluid

Corrected simulated body fluid (c-SBF) was prepared by procedure described by Kokubo and Takadama [10]. As prepared c-SBF contains 142 mM  $\text{Na}^+$ , 5 mM  $\text{K}^+$ , 1.5 mM  $\text{Mg}^{2+}$ , 2.5 mM  $\text{Ca}^{2+}$ , 147.8 mM  $\text{Cl}^-$ , 4.2 mM  $\text{HCO}_3^-$ , 1.0 mM  $\text{M}_4^{2-}$  and 0.5 mM  $\text{SO}_4^{2-}$ . 10 mg of Mg- and Mg, Si- substituted HAP were added in 2 mL Eppendorf tubes along with 1 mL of c-SBF. The tubes were kept in thermostated water bath at 37°C for 28 days. The c-SBF was exchanged every day by centrifugation and decantation. The solid phase was filtered after 1, 14 and 28 days through 0.45  $\mu\text{m}$

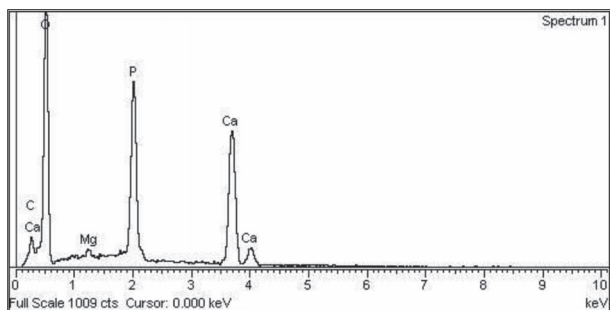
Millipore filter paper, washed 3 times with MilliQ water, one time with ethanol, and dried in nitrogen gas flow. The samples were kept in desiccator until further analysis.

### Characterization methods

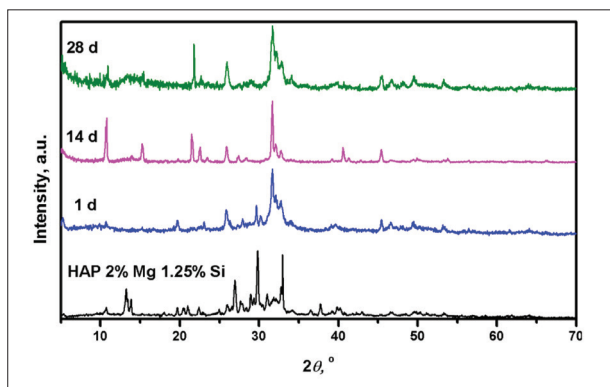
XRD patterns were recorded on Panalytical AERIS Research Edition in Bragg – Brentano geometry using CuK $\alpha$  radiation in angular scan range from 5° to 70°  $2\theta$  using a step size of 0.02°  $2\theta$  and a scan rate of 1°  $\text{min}^{-1}$ .

FTIR spectra of the samples were recorded on an FTIR spectrometer equipped with an attenuated total reflection module (Tensor I, Bruker, Ettlingen, Germany) in the range from 4000–400  $\text{cm}^{-1}$ , with a resolution of 1  $\text{cm}^{-1}$ . The presented spectra are the average of 16 scans.

The morphology of the solid phases was determined by a field emission scanning electron microscope (FE-SEM; JEOL JSM-7000F microscope). For SEM analysis a required amount of powder was placed on a sample holder covered with carbon glue and the excess powder was removed by gentle nitrogen gas flow.

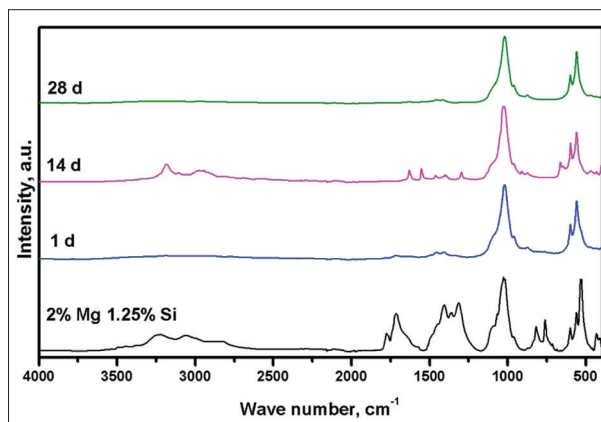


**Figure 4.** EDS spectrum of HA doped with 2% Mg  
**Slika 4.** EDS spektar HA dopiranog sa 2% Mg



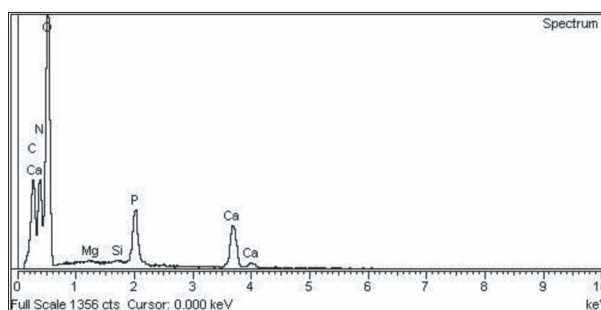
**Figure 5.** XRD patterns of HA doped with 2% Mg and 1.25% Si: the initial powder and powder obtained after immersion in corrected simulated body fluid for 1 - 28 days at 37°C.

**Slika 5.** XRD spektri HA dopiranog sa 2% Mg i 1,25% Si: početni prah i prahovi dobijeni posle potapanja u korigovani simulirani telesni fluid tokom 1–28 dana na 37°C.

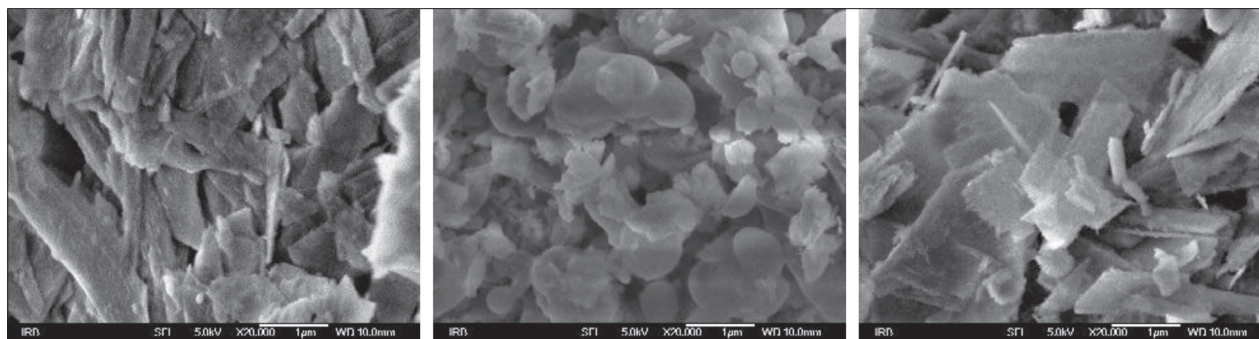


**Figure 6.** FTIR spectra of HA doped with 2% Mg and 1.25% Si: the initial powder and powder obtained after immersion in corrected simulated body fluid for 1–28 days at 37°C

**Slika 6.** FTIR spektri HA dopiranog sa 2% Mg i 1,25% Si: početni prah i prahovi dobijeni posle potapanja u korigovani simulirani telesni fluid tokom 1–28 dana na 37°C



**Figure 8.** EDS spectrum of HA doped with 2% Mg and 1.25% Si  
**Slika 8.** EDS spektar HA dopiranog sa 2% Mg i 1,25% Si



**Figure 7.** SEM images of HA doped with 2% Mg and 1.25% Si: a) the initial powder, b) powder after immersion in corrected simulated body fluid for 14 days, c) powder after immersion in corrected simulated body fluid for 28 days

**Slika 7.** SEM slike HA dopiranog sa 2% Mg i 1,25% Si: a) početni prah, b) prah posle potapanja u korigovani simulirani telesni fluid tokom 14 dana, c) prah posle potapanja u korigovani simulirani telesni fluid tokom 28 dana

## RESULTS AND DISCUSSION

### Mg-substituted HAP

XRD analysis revealed the peaks characteristic for HA (Figure 1); most intensive peaks can be observed at  $2\theta$  25.90°, 29.01°, 31.19°, 32.17° and 32.95° corresponding to planes (002), (210), (211), and (112), respectively. In addition to HA peaks, a low intensity peak at 37.71° characteristic for  $\text{Mg}(\text{OH})_2$  was observed [11].

After immersion in SBF, peaks corresponding to (110), (111), (210), (112) and (310) HA reflections, as well as

$\text{Mg}(\text{OH})_2$  peak were not detected, already after 1 day (Figure 1). The low intensity maximum in  $2\theta$  range 12–15° was observed after 14 days which was not observed in Mg-HA. In addition, low intensity peaks at around 45.5° and 56.5°, not present in the initial Mg-HA powder, were also observed.

FTIR spectra (Figure 2) showed vibrational bands of phosphate and hydroxyl groups characteristic for HA. The vibration of phosphate groups were observed at 1092, 1022, 961, 600, 561 and 472  $\text{cm}^{-1}$  [12]. Besides,  $\text{H}_2\text{O}$  bands were observed at 3564–3000  $\text{cm}^{-1}$  and 1633  $\text{cm}^{-1}$ .  $\text{CO}_3^{2-}$  bands at 1426, 1321 and 873  $\text{cm}^{-1}$  [11, 13], and  $\text{P}_2\text{O}_7^{4-}$  band



at 715 and 828  $\text{cm}^{-1}$ . The presence of water band in region 3564 – 3000  $\text{cm}^{-1}$ , indicates the higher strength with which Mg-HA surfaces bind water molecules [14]. Two possible sites that Mg can occupy are the two crystallographic calcium sites. However, the majority of theoretical and experimental studies confirm that Mg preferentially occupies M(II) site [15].

FTIR spectra of the samples immersed in SBF did not contain water vibration band in region 3564 – 3000  $\text{cm}^{-1}$  and band at 1633  $\text{cm}^{-1}$ , as well as band at 828  $\text{cm}^{-1}$  and  $\text{P}_2\text{O}_7^{4-}$  band at 715  $\text{cm}^{-1}$  present in spectra of non-treated Mg-HA (Fig.2). Also, the intensity of  $\text{CO}_3^{2-}$  bands in region 1450 – 1330  $\text{cm}^{-1}$  decreased.

SEM micrograph revealed formation of aggregated leaf-like crystals (Figure 3), while EDS spectra confirmed the presence of the magnesium in the material (Figure 4). The leaf like crystals appeared more developed after immersion in SBF for 14 and 28 days (Figure 3b and c).

### Mg, Si- substituted HA

The XRD pattern (Figure 5) indicated formation of the mixture of HA with small amounts of monetite ( $\beta\text{-Ca}_3\text{PO}_4$ ) and magnesium phosphate (MP,  $\text{Mg}_7(\text{PO}_4)_2(\text{OH})_8$ ). All the most intense HAP peaks were present in the diffraction pattern, while only the most intense peak and several low intensity peaks of  $\beta\text{-Ca}_3\text{PO}_4$  were observed, as well as one peak of MP.

Upon immersion in SBF already after 1<sup>st</sup> day, only peaks characteristic of HA were seen and a single peak of MP. After 14 days peaks were becoming narrower while after 28 days the broadening of the peaks was observed which can be a consequence of precipitation of additional material from SBF.

FTIR spectra (Figure 6) showed vibrational bands of phosphate groups at 1089, 1023, 962, 600, 560 and 532  $\text{cm}^{-1}$ . The intensity of water band in region 3500 – 3000  $\text{cm}^{-1}$  increased compared to Mg-HA. Fading of  $\text{OH}^-$  bands at about 3570  $\text{cm}^{-1}$  and 630  $\text{cm}^{-1}$  which are typically present in FTIR spectrum of HA was explained by reduction of the number of  $\text{OH}^-$  groups in order to maintain charge balance due to  $\text{PO}_4^{3-}$  substitution with  $\text{SiO}_4^{4-}$  [16]. This also confirms partial incorporation of  $\text{SiO}_4^{4-}$  into the HA [17]. In addition, vibrations of Si-O-Si group were detected at 1067  $\text{cm}^{-1}$  and 820  $\text{cm}^{-1}$  [18] and vibrations of Si-O group at 761  $\text{cm}^{-1}$  and 432  $\text{cm}^{-1}$  [17]. In FTIR spectra, after immersion in SBF, the water band in range 3500 – 3000  $\text{cm}^{-1}$  and bands in range 1800 – 1500  $\text{cm}^{-1}$ , 1500 – 1100  $\text{cm}^{-1}$  and 810 – 650  $\text{cm}^{-1}$  were faded.

SEM micrography revealed irregular, thin plate-like crystals (Figure 7a), while EDS spectra confirmed the presence of the magnesium and silicon in the material (Figure 8). After 14 days immersion in SBF there was no significant morphological changes (Figure 7b). However after 28 days, growth of thin leaf-like crystals on the sample surface was observed (Figure 7c).

### CONCLUSION

Mg- and Si- substituted HA attract attention as biomimetic and bioactive hard tissue regeneration materials since they show beneficial biological effects. In order to determine their true potential for biomedical application their properties have to be carefully investigated. Despite the increasing number of studies of Mg- and Si- substituted HAs, some topics are rarely discussed although they are of huge importance for successful application of implant materials, such as the stability of material in model media.

In this study, behaviour of Mg- and Si-substituted HA upon immersion in simulated body fluid was investigated which mimics physiological conditions. The structure and composition of these materials were investigated by XRD, FTIR and SEM and the obtained results indicated that they have good potential for biomedical applications.

### ACKNOWLEDGEMENT

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# Hidroksiapatit supstituisan magnezijumom i silicijumom: ponašanje u simuliranom telesnom fluidu

Božana Petrović<sup>1</sup>, Maja Krstić<sup>1</sup>, Tihana Mudrinić<sup>2</sup>, Maria Čebela<sup>1</sup>, Maja Dutour-Sikirić<sup>3</sup>

<sup>1</sup>Institut za nuklearne nauke Vinča – Institut od nacionalnog značaja za Republiku Srbiju, Univerzitet u Beogradu, Beograd, Srbija;

<sup>2</sup>Institut za hemiju, tehnologiju i metalurgiju – Institut od nacionalnog značaja za Republiku Srbiju, Univerzitet u Beogradu, Beograd, Srbija;

<sup>3</sup>Institut „Ruđer Bošković“, Zagreb, Hrvatska

## KRATAK SADRŽAJ

Zbog svoje sličnosti sa biološkim apatitom koji se nalazi u tvrdim tkivima kičmenjaka, kalcijum-hidroksiapatit je jedan od najistraživanijih materijala u inženjerstvu koštanog tkiva. Pošto biološki apatit nije stehiometrijski, jonski supstituisani hidroksiapatiti privlače veliku pažnju jer više oponašaju sastav prirodne kosti. Iako postoje mnoga istraživanja uticaja stranih jona na strukturu i fizičko-hemijske osobine jonski supstituisanih hidroksiapatita, nema mnogo podataka o njihovom ponašanju u različitim medijumima.

U ovom radu, magnezijum, koji igra ključnu ulogu u metabolizmu kostiju, i silicijum, koji je neophodan za normalan razvoj skeleta, korišćeni su kao joni supstituenti. Ponašanje Mg i Si supstituisanog hidroksiapatita u simuliranom telesnom fluidu je ispitivano pomoću XRD, FTIR i SEM. Dobijeni rezultati su potvrdili veliki potencijal ovih supstituisanih hidroksiapatita za biomedicinsku primenu.

**Ključne reči:** supstituisani hidroksiapatit; magnezijum; silicijum; SBF

## UVOD

Pošto kalcijum-fosfati čine glavnu mineralnu komponentu tvrdih tkiva kičmenjaka, njihovi sintetički analozi su najčešće korišćeni materijali u ortopediji i stomatologiji [1, 2, 3]. Naime, mineralna faza kostiju i zuba je bazični kalcijum-fosfat, koji se izjednačava sa sintetičkim kalcijum-hidroksiapatitom ( $\text{HA}$ ,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) [2]. HA ispoljava odličnu biokompatibilnost i bioaktivnost zbog svoje sličnosti sa neorganskim fazama biomineralizovanih tkiva, što ga čini najproučavanijim kalcijum-fosfatom [1–5]. Koristi se za brojne biomedicinske primene, uglavnom u inženjerstvu koštanog tkiva, kao što su: koštani nosač, za restauraciju koštanih i parodontalnih defekata, kao premaz na metalnim implantatima, sistem za isporuku lekova i antibakterijsko sredstvo.

Uprkos velikoj sličnosti, biološki apatiti se razlikuju od stehiometrijskog HA u nekoliko aspekata, kao što su nestehiometrija, male veličine kristala i loša kristaliničnost, što su važni faktori povezani sa relativno visokom rastvorljivošću bioloških apatita u poređenju sa stehiometrijskim HA [2]. Biološki apatiti su zapravo karbonizovani apatiti koji sadrže i strane jone, koji se mogu ugraditi ili adsorbovati na površini kristala apatita. Takođe, nukleacija i rast kalcijum-fosfata u biološkim sistemima dešavaju se u okruženju bogatom jonima, što može uticati na kinetiku kristalizacije i dalje na njihovu relativnu stabilnost. Stoga je proučavanje jonske supstitucije u HA značajno iz više razloga: boljeg razumevanja procesa biomineralizacije, kontrole svojstava precipitirane faze, povećanja bioaktivnosti materijala i isporuke jona za lečenje obolele kosti [2, 6].

Magnezijumovi i silicijumovi joni su dobri kandidati za ugradnju u rešetku HA zbog svog biološkog značaja. Kao što je poznato, magnezijum je važan element u tragovima u kostima i zubima, a njegov nedostatak uzrokuje krtošću kostiju [2, 7]. Ima ključnu ulogu u metabolizmu kostiju, jer utiče na aktivnost osteoblasta i osteoklasta, a samim tim i na rast kostiju. Silicijum ima metaboličku ulogu u rastu kosti i navodi se kao neophodan element u tragovima za normalan razvoj skeleta, posebno u početnim fazama formiranja kostiju [3, 6, 8]. Pored

toga, predložena je simultana supstitucija  $\text{Mg}/\text{SiO}_2$  jona kao način dobijanja materijala sličnog biološkom apatitu [2]. Zbog toga su ova dva elementa izabrana da zamene  $\text{Ca}^{2+}/\text{PO}_4^{3-}$  u rešetki HA i da se istraži njihovo ponašanje nakon potapanja u simulirani telesni fluid.

## MATERIJAL I METODE

### Sinteza supstituisanog hidroksiapatita

Sve korišćene hemikalije kupljene su od Sigma Aldrich, Nemačka.

Mg-supstituisani HA je sintetisan dodavanjem rastvora  $(\text{NH}_4)_2\text{HPO}_4$  kap po kap u rastvor  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  i  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ . Reakciona smeša je zagrevana na  $100^\circ\text{C}$  pod reflusom, pet sati. Tokom zagrevanja dodavane su urea i ureaza i postignut je pH 8,8. Proizvod je filtriran, ispran dejonizovanom vodom i etanolom i osušen na  $120^\circ\text{C}$ .

Mg, Si supstituisani HA je sintetisan dodatkom rastvora koji sadrži  $(\text{NH}_4)_2\text{HPO}_4$  i sol  $\text{SiO}_2$  na kontrolisan način u rastvor  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  i  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ . Sol  $\text{SiO}_2$  je sintetizovan postupkom koji su opisali Jokanović i sar. [9]. Reakciona smeša je zagrevana na  $100^\circ\text{C}$ , pod reflusom, pet sati. Tokom zagrevanja dodavane su urea i ureaza i postignut je pH 7,9. Proizvod je filtriran, ispran dejonizovanom vodom i etanolom i osušen na  $200^\circ\text{C}$ .

### Mineralizacija u simuliranom telesnom fluidu

Korigovani simulirani telesni fluid (c-SBF) pripremljen je postupkom koji su opisali Kokubo i Takadama [10]. Tako pripremljeni c-SBF sadrži 142 mM  $\text{Na}^+$ , 5 mM  $\text{K}^+$ , 1,5 mM  $\text{Mg}^{2+}$ , 2,5 mM  $\text{Ca}^{2+}$ , 147,8 mM  $\text{Cl}^-$ , 4,2 mM  $\text{HCO}_3^-$ , 1,0 mM  $\text{HPO}_4^{2-}$  i 0,5 mM  $\text{SO}_4^{2-}$ .

Deset mg Mg- i Mg, Si supstituisanog HAP-a je dodato u Ependorfov epruvetu od 2 mL zajedno sa 1 mL c-SBF. Epruvete su držane u termostatanom vodenom kupatilu na  $37^\circ\text{C}$  tokom

28 dana. C-SBF se menjao svakog dana centrifugiranjem i dekantacijom. Čvrsta faza je filtrirana posle 1, 14 i 28 dana kroz 0,45 µm filter papir Millipore, isprana tri puta vodom Milli Q, jednom sa etanolom, i osušena u struji azota. Uzorci su čuvani u eksikatoru do dalje analize.

### Metode karakterizacije

XRD spektri su snimljeni na Panalytical Aeris Research Edition u Bragg–Brentano geometriji korišćenjem zračenja CuKα u opsegu ugaonog skeniranja  $2\theta$  od  $5^\circ$  do  $70^\circ$  koristeći veličinu koraka od  $0,02^\circ 2\theta$  i brzinu skeniranja od  $1^\circ \text{ min}^{-1}$ .

FTIR spektri uzoraka su snimljeni na FTIR spektrometru opremljenom modulom ometane ukupne refleksije (Tensor I, Bruker, Ettlingen, Nemačka) u opsegu od  $4000\text{--}400 \text{ cm}^{-1}$ , sa rezolucijom od  $1 \text{ cm}^{-1}$ . Prikazani spektri su proseki od 16 skeniranja.

Morfologija čvrstih faza određena je emisijom skenirajućim elektronskim mikroskopom (FE-SEM; JEOL JSM-7000F mikroskop). Za SEM analizu potrebna količina praha je stavljena na držač uzorka prekriven ugljeničnim lepkom i višak praha je uklonjen blagim protokom gasa azota.

## REZULTATI I DISKUSIJA

### Mg-supstituisani HAP

XRD analiza je otkrila pikove karakteristične za HA (Slika 1); najintenzivniji pikovi se mogu uočiti na  $2\theta$   $25,90^\circ$ ,  $29,01^\circ$ ,  $31,19^\circ$ ,  $32,17^\circ$  i  $32,95^\circ$  koji odgovaraju ravnima (002), (210), (211) i (112), respektivno. Pored HA pikova, primećen je i pik niskog intenziteta na  $37,71^\circ$  karakterističan za  $\text{Mg}(\text{OH})_2$  [11].

Nakon potapanja u SBF, pikovi koji odgovaraju (110), (111), (210), (112) i (310) refleksijama HA, kao i  $\text{Mg}(\text{OH})_2$  pik nisu detektovani već nakon jednog dana (Slika 1). Maksimum niskog intenziteta u opsegu  $2\theta$   $12\text{--}15^\circ$  primećen je posle 14 dana, što nije primećeno kod izvornog Mg-HA. Pored toga, primećeni su i pikovi niskog intenziteta na oko  $45,5^\circ$  i  $56,5^\circ$ , koji nisu prisutni u početnom Mg-HA prahu.

FTIR spektri (Slika 2) pokazuju vibracione trake fosfatnih i hidroksilnih grupa karakterističnih za HA. Vibracije fosfatnih grupa su primećene na  $1092$ ,  $1022$ ,  $961$ ,  $600$ ,  $561$  i  $472 \text{ cm}^{-1}$  [12]. Osim toga, trake  $\text{H}_2\text{O}$  trake su primećene na  $3564\text{--}3000 \text{ cm}^{-1}$  i  $1633 \text{ cm}^{-1}$ ;  $\text{CO}_3^{2-}$  trake na  $1426$ ,  $1321$  i  $873 \text{ cm}^{-1}$  [11, 13] i  $\text{P}_2\text{O}_7^{4-}$  trake na  $715$  i  $828 \text{ cm}^{-1}$ . Prisustvo vodenog pojasa u regionu  $3564\text{--}3000 \text{ cm}^{-1}$  ukazuje na veću snagu kojom površina Mg-HA vezuje molekule vode [14]. Dva moguća mesta koja Mg može zauzeti su dva kristalografska mesta kalcijuma. Međutim, većina teorijskih i eksperimentalnih studija potvrđuje da Mg prvenstveno zauzima M(II) mesto [15].

FTIR spektri uzoraka uronjenih u SBF nisu sadržali vibracioni opseg vode u oblasti  $3564\text{--}3000 \text{ cm}^{-1}$  i traku na  $1633 \text{ cm}^{-1}$ , kao ni traku na  $828 \text{ cm}^{-1}$  i  $\text{P}_2\text{O}_7^{4-}$  traku na  $715 \text{ cm}^{-1}$  koja je prisutna u spektru netretiranog Mg-HA (Slika 2). Takođe, smanjen je intenzitet  $\text{CO}_3^{2-}$  traka u regionu  $1450\text{--}1330 \text{ cm}^{-1}$ .

SEM snimci su pokazali formiranje agregiranih kristala nalik na listove (Slika 3a), dok je EDS spektar potvrdio prisustvo

magnezijuma u materijalu (Slika 4). Kristali nalik na listove su izgledali razvijeniji posle potapanja u SBF tokom 14 i 28 dana (slike 3b i 3c).

### Mg, Si- supstituisani HA

XRD spektar (Slika 5) ukazuje na formiranje smeše HA sa malim količinama monetita ( $\beta\text{-Ca}_3\text{PO}_4$ ) i magnezijum-fosfata ( $\text{Mg}_7(\text{PO}_4)_2(\text{OH})_8$ ). Svi najintenzivniji pikovi HA su bili prisutni na difraktogramu, dok su uočeni samo najintenzivniji pik i nekoliko pikova niskog intenziteta koji pripadaju  $\beta\text{-Ca}_3\text{PO}_4$ , kao i jedan pik MP.

Nakon potapanja u SBF, već nakon prvog dana, uočeni su samo pikovi karakteristični za HA i jedan pik MP. Nakon 14 dana pikovi su postajali sve uži, dok je nakon 28 dana primećeno proširenje pikova, što može biti posledica taloženja dodatnog materijala iz SBF-a.

FTIR spektri (Slika 6) pokazali su vibracione trake fosfatnih grupa na  $1089$ ,  $1023$ ,  $962$ ,  $600$ ,  $560$  i  $532 \text{ cm}^{-1}$ . Intenzitet vodenog pojasa u regionu  $3500\text{--}3000 \text{ cm}^{-1}$  je povećan u poređenju sa Mg-HA. Iščezavanje OH- traka na oko  $3570 \text{ cm}^{-1}$  i  $630 \text{ cm}^{-1}$  koje su tipično prisutne u FTIR spektru HA objašnjeno je smanjenjem broja OH- grupa kako bi se održao balans naboja zbog zamene  $\text{PO}_4^{3-}$   $\text{SiO}_4^{4-}$  jonima [16]. Ovo takođe potvrđuje delimičnu inkorporaciju  $\text{SiO}_4^{4-}$  u HA [17]. Pored toga, detektovane su vibracije grupe Si-O-Si na  $1067 \text{ cm}^{-1}$  i  $820 \text{ cm}^{-1}$  [18] i vibracije grupe Si-O na  $761 \text{ cm}^{-1}$  i  $432 \text{ cm}^{-1}$  [17]. U FTIR spektrima, nakon potapanja u SBF, vodeni pojas u opsegu  $3500\text{--}3000 \text{ cm}^{-1}$  i trake u opsegu  $1800\text{--}1500 \text{ cm}^{-1}$ ,  $1500\text{--}1100 \text{ cm}^{-1}$  i  $810\text{--}650 \text{ cm}^{-1}$  iščezli su.

SEM mikrografijom su otkriveni nepravilni, tanki pločasti kristali (Slika 7a), dok je EDS spektar potvrdio prisustvo magnezijuma i silicijuma u materijalu (Slika 8). Posle 14 dana potapanja u SBF nije bilo značajnih morfoloških promena (Slika 7b). Međutim, nakon 28 dana primećen je rast tankih lisnatih kristala na površini uzorka (Slika 7c).

## ZAKLJUČAK

Mg- i Si- supstituisani HA privlače pažnju kao biomimetični i bioaktivni materijali za regeneraciju tvrdih tkiva jer pokazuju korisne biološke efekte. Da bi se utvrdio njihov pravi potencijal za biomedicinsku primenu, njihova svojstva moraju biti pažljivo istražena. Uprkos sve većem broju studija o Mg- i Si- supstituisanim HA, o nekim temama se retko diskutuje iako su od ogromnog značaja za uspešnu primenu implantnih materijala, kao što je stabilnost materijala u modelnim medijumima.

U ovoj radu ispitivano je ponašanje Mg- i Si-supstituisanog HA pri uranjanju u simulirani telesni fluid koji imitira fiziološke uslove. Struktura i sastav ovih materijala su ispitivani pomoću XRD, FTIR i SEM i dobijeni rezultati su pokazali da imaju dobar potencijal za biomedicinsku primenu.

### Napomena

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