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IN VITRO HYDROXYAPATITE FORMING ABILITY OF DICALCIUM SILICATE PREPARED FROM LOCAL RAW MATERIALS AND SINTERED AT 1050 °C

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In this study, Ca_2SiO_4 powders were synthesised using solid state reaction. The starting powders were sintered at 1050 °C for 2 h. Moreover, the in vitro bioactivity of $\beta\text{-Ca}_2\text{SiO}_4$ was investigated by soaking the powders in a simulated body fluid (SBF) for various time periods to analyse the growth of hydroxyapatite (HA) on the surface of these powders. The synthesised powders were characterised by scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform infrared (FTIR), and Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) analysis. The simulated body fluid results showed that the $\beta\text{-Ca}_2\text{SiO}_4$ powders had good bioactivity to induce hydroxyapatite formation on their surface. The results obtained showed that (HCA) can be formed on the surface of $\beta\text{-Ca}_2\text{SiO}_4$ soaked in the SBF solution for 1 day, and a continuous layer of dense HCA deposits covered the surface of $\beta\text{-Ca}_2\text{SiO}_4$ powders after 3 days of soaking in the SBF solution. Finally, the results obtained suggest that $\beta\text{-Ca}_2\text{SiO}_4$ ceramics are promising candidates for bone regeneration.

INTRODUCTION

An increasing number of bone defects caused by trauma or inflammation has fueled the huge demand for bone grafting materials for use in orthopaedic surgery [1, 2]. Bioactive materials can bring out a particular biological response at the interface between the tissue and the materials which results in the formation of a bond between the tissues and the materials. The formation of a biologically carbonate-containing hydroxyapatite layer (chemically and crystallographically equivalent to the mineral phase in a bone) on an implant is due to the ion-exchange reaction between the implant and the surrounding body fluids (SBFs) which promote the connection between the natural tissues and the material [3].

Since the discovery of bioglass and bioceramics by Hench and co-workers in 1970 [4], various types of and glass-ceramics, such as bioactive glasses [5-8], and apatite/wollastonite glass ceramics [9-13], have been viewed as bioactive biomaterials for tissue repair and replacement [14-16].

Calcium phosphate cements (CPCs), due to their good self-setting and biocompatibility, have generally been used as clinical bone implants [17]. Indeed, a previous study has shown that their degradation tends to be slow [18].

Recently, various studies have shown that silicon containing biomaterials show good bioactivity and have the potential to enhance osteo-genesis [19-23]. Silicate has shown its excellence in osseous tissue repair in in vitro and in vivo tests [18, 24-28]. Furthermore, Ohtsuki et al [29], showed that calcium ions dissolved from CaO in SiO₂-based glasses increase the degree of saturation of the simulated body fluid (SBF) and the hydrated silica formed on their surface provides favourable sites for apatite nucleation. Moreover, Li et al [30], applied a ‘charged surface’ theory for the explanation of the reaction of bioactive glass in a simulated body fluid and indicated that precipitation of apatite on the surface of these bioactive glass in the SBF is due to the formation of an electric double layer between the bioactive glass and the solution. Therefore, some ceramics, such as dicalcium silicate (Ca_2SiO_4), are regarded as biomaterials

and they may be potential candidates for artificial bone replacement [31].

Furthermore, there are many Ca_2SiO_4 preparation process from SiO_2 and CaO oxides, such as the sol-gel method. However, because of their complicated sample preparation, another method is proposed, which is based on replacing expensive materials by other less expensive raw materials which are abundantly available in the world. Many works have been published with regards to the valorisation of native raw materials within bioceramic applications [32-36].

Consequently, the main goal of this study is to evaluate the bioactivity of pure $\beta\text{-Ca}_2\text{SiO}_4$ (prepared from local raw materials) by examining the hydroxyapatite (HA) formation on their surface in an SBF solution.

EXPERIMENTAL

Preparation of powders

Ca_2SiO_4 powders were synthesised through a solid-state reaction method according to our previous study [34]. A mixture of high purities, SiO_2 and CaO , extracted from local CaCO_3 (CaCO_3 was calcined at 900 °C for 9 hours (h) then hydrated by distilled water and then followed by a second calcination at 800 °C for 2 h). The purity of the obtained CaO was about 99.7 %, using fluorescence XRD analysis. As reported by Ming et al [37], this raw material has a high-purity calcium oxide ($\text{CaO} \geq 99.0\%$).

CaO and SiO_2 with a CaO/SiO_2 molar ratio equal to two were mixed using wet milling for 3 h, which was then calcined at 650 °C for 2 hours. Subsequently, the powder mixtures were sintered at 1050 °C for 2 hours.

In vitro tests (apatite forming ability)

The in vitro properties of the sintered specimens were investigated by their apatite forming ability in the simulated body fluid (SBF). The powders sintered at 1050 °C for 2 h were soaked in an SBF solution whose ion concentration was nearly equal to those of human body blood plasma, as shown in Table 1 [38]. The SBF solution was buffered at $\text{pH} = 7.4$ with trimethanol aminomethane-HCl-6N. The powders were immersed in the SBF solution at 37 °C for 4 hours and for 1, 3, 7, 15 days. After soaking for various periods, the samples were removed from the fluids with deionised water, and dried at room temperature.

Characterisations

The phase identification and morphology of the powders before and after soaking in the SBF solutions were characterised by X-ray diffraction (XRD) (BRUKER, D8 ADVANCE) (Karlsruhe, Germany) with $\text{CuK}\alpha$ radiation ($\lambda = 0.154\text{ nm}$) and an Ni filter, with a working voltage of 40 kV and a working current of 30 mA, Fourier-transform infrared (FTIR) spectroscopy (Bruker Equinox 55: $4000\text{-}400\text{ cm}^{-1}$). The surfaces of the powders were observed by Scanning Electron Microscopy (SEM) (HITACHI, JSM-6301 F) (Tokyo, Japan) working at 7 kV as the accelerating voltage and by Energy Dispersive Spectrometry (EDS) (JEOL JSM-6400). The changes in the concentrations of the Ca, P and Si ions in the SBF solution after soaking were measured by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (Spectro Ciros Vision, Karlsruhe, Germany).

RESULTS AND DISCUSSION

Characterisation of the $\beta\text{-Ca}_2\text{SiO}_4$ powders

Figure 1 (0 hours) shows the XRD patterns of the dicalcium silicate powders sintered at 1050 °C for 2 h before soaking in the SBF solution. The XRD spectrum demonstrates the formation of the $\beta\text{-Ca}_2\text{SiO}_4$ phase (JCPDS card number 72-1130). The peak positions are in good agreement with those of reference [39].

The FTIR spectra of the dicalcium silicate powders sintered at 1050 °C and before soaking in the SBF are illustrated in Figure 2 (0 hour). It is obvious that the spectrum of the dicalcium silicate powder was dominated by the Si-O vibrations. The peaks at 472, and 902 cm^{-1} are due to the Si-O vibration and the peaks mainly occur in the range of $519\text{--}994\text{ cm}^{-1}$ are due to the O-Si-O vibration [40, 41]. Moreover, an OH^{-1} absorption band around a 3500 cm^{-1} can be seen in this spectrum.

Apatite-formation ability of the $\beta\text{-Ca}_2\text{SiO}_4$ powders

Figure 1 shows the XRD patterns of the $\beta\text{-Ca}_2\text{SiO}_4$ powders before and after soaking in the SBF solution for various time periods. It is obvious that the characteristic peak intensities of $\beta\text{-Ca}_2\text{SiO}_4$ decrease with an increase in the soaking time and disappeared after 1 day of soaking. New peaks for CaCO_3 were apparent after 1 day (the JCPDS card number for CaCO_3 is 88-1807),

Table 1. Ion concentration of the SBF in comparison to human blood plasma.

Types	Ion concentrations [mM]							
	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
SBF	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5
Blood plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5

while the characteristic peaks of HA in $2\theta = 32$ and 26 appeared after 1 day of soaking, indicating the formation.

The FTIR spectra of the powders sintered at 1050°C before and after soaking in the SBF are displayed in Figure 2. The IR spectrum of the $\beta\text{-Ca}_2\text{SiO}_4$ powders is also given in this figure, showing the spectral characteristics of $\beta\text{-Ca}_2\text{SiO}_4$. When the soaking time increased, the intensity of the silicate absorption bands decreased. Simultaneously, new absorption bands at 566 and 603 cm^{-1} and a broad band appeared after 1 day of soaking, and these bands were split from the P–O bending vibration (ν_4) of the PO_4^{3-} group around 598 cm^{-1} . The band around 1100 cm^{-1} could be attributed to the P–O stretching vibration (ν_3) mode [42]. According to the report of IR correlation charts [43], all these bands were characteristic of HA crystals, which indicated the formation of HA on the surface of the $\beta\text{-Ca}_2\text{SiO}_4$ powders. A broad OH[−] absorption band around 3500 cm^{-1} can be seen in these spectra. Furthermore, the C–O stretching of the CO_3^{2-} groups at 872 , 1419 and 1490 cm^{-1} were observed after 1 day of soaking, which indicated that HCA was formed on the surface of the $\beta\text{-Ca}_2\text{SiO}_4$ powders after soaking in the SBF solution. These results further confirmed that a carbonate-containing hydroxyapatite layer could be induced to deposit on the surface of $\beta\text{-Ca}_2\text{SiO}_4$ ceramics in the SBF of HA on the surface of the $\beta\text{-Ca}_2\text{SiO}_4$ powders.

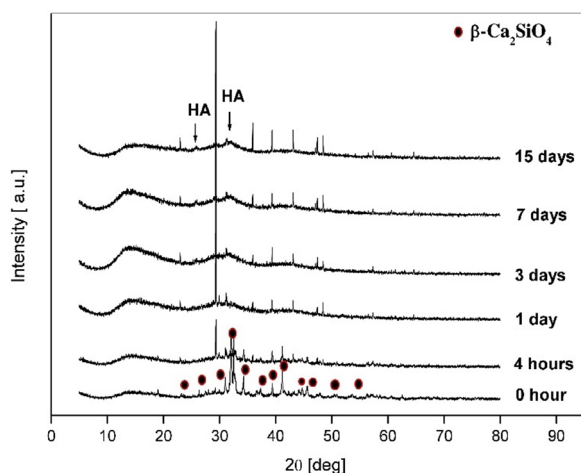


Figure 1. XRD patterns of the $\beta\text{-Ca}_2\text{SiO}_4$ powders sintered at 1050°C for 2 hours before and after soaking in the SBF solution for various periods.

The surface morphology of the $\beta\text{-Ca}_2\text{SiO}_4$ powders before and after soaking in the SBF solution for 4 hours, and 1, 3, 7 and 15 days are presented in Figure 3 and Table 2. In comparison with the particles before soaking in the SBF solution presented in Figure 3a, small ball-like particles appeared on the surface of the $\beta\text{-Ca}_2\text{SiO}_4$ powders soaked in the SBF for 4 h as shown Figure 3b. Up to 1 day of soaking, ball-like particles were observed

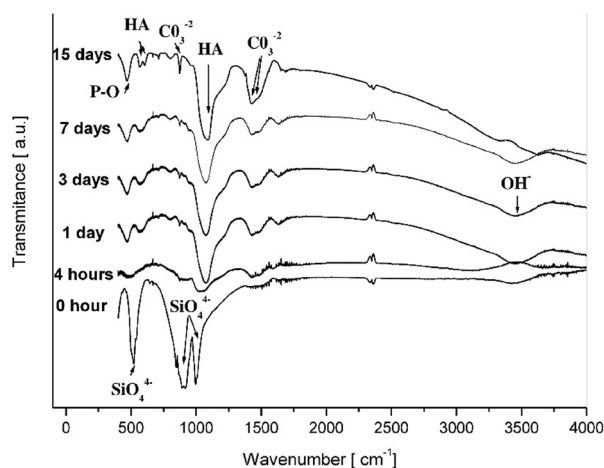


Figure 2. FTIR spectra of the $\beta\text{-Ca}_2\text{SiO}_4$ powders sintered at 1050°C for 2 h before and after soaking in the SBF solution for various periods.

inside the pores and on the surface of the $\beta\text{-Ca}_2\text{SiO}_4$ powders (Figure 3c). After prolonged soaking for up to 3 days, the surface morphology of the powders changed and a continuous layer of dense deposits formed on the surface of the $\beta\text{-Ca}_2\text{SiO}_4$ powders as given in Figures 3 (d, e, f). The results are in good agreement with previous studies [39].

Table 2. EDS quantitative analysis (element concentration) of the $\beta\text{-Ca}_2\text{SiO}_4$ powders after soaking in the SBF solution for 15 days.

Element	% Mass	% Atomic
Si K	7.43	37.30
P K	4.91	22.39
Ca K	11.45	40.31
Total	23.79	

Table 2 shows the EDS quantitative analysis (elements concentration) of the powders after 15 days of soaking in the SBF solution. The EDS quantitative analysis of the continuous layer formed on the surface of powders soaked in the SBF for 15 days gave a Ca/P ratio of around 1.80 which is slightly higher than that in hydroxyapatite. This fact suggests that HCA is formed on the surface of the $\beta\text{-Ca}_2\text{SiO}_4$ powders (a Ca/P ratio value greater than the pure hydroxyapatite) according to the results reported by other authors [44–46]. The HCA formation is a consequence of the usually high levels of calcium in the solution near the surface, due to dissolution of $\beta\text{-Ca}_2\text{SiO}_4$, and the enrichment with the carbonate ions present in the SBF that give rise to the HCA on the surface.

The formation mechanism of apatite was proposed by Hench et al [47]. The exchange of calcium ions in ceramics with H^+ in an SBF solution gives rise

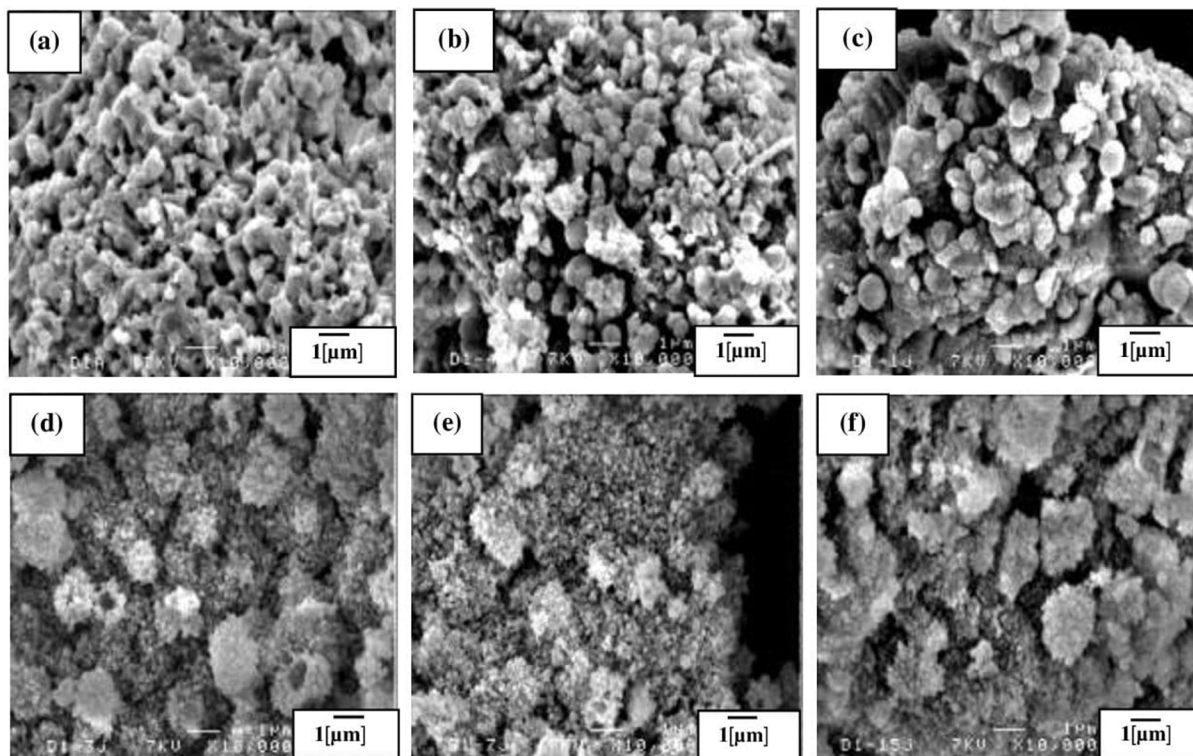


Figure 3. SEM micrographs of the β - Ca_2SiO_4 powders sintered at 1050 °C for 2 h before and after soaking in the SBF solution for various periods; (a) 0 h, (b) 4 h, (c) 1 d, (d) 3 d, (e) 7 d, (f) 15 d.

to the formation of silanol (Si-OH) in the surface layer, an increase in the pH value at the ceramics-SBF interface, and eventually the production of a negatively charged surface with the functional group (Si-O⁻). This later had been proposed to be a catalysing agent and could provide specific favourable sites for the apatite nucleation [48, 49].

Concentration changes of Ca, P and Si in the SBF solution

Figure 4 shows the changes in the concentrations of Ca, P and Si in the SBF solutions measured by ICP after soaking for various time periods. It is obvious that the ion concentrations of the SBF solutions changed markedly after soaking in the SBF solution. The Ca and Si concentrations in the SBF increased with an increase in the soaking time up to 1 day. Afterwards, the Ca concentration in the SBF decreased after 1 day of soaking. In contrast to the increase in the Ca and Si concentration, the P concentration of the SBF solutions decreased during the soaking periods.

The increases in the calcium and silicon concentrations were attributed to the dissolution of these ions from the β - Ca_2SiO_4 powders. Moreover, the decrease in the calcium ions after 1 day of soaking is due to the faster consumption of Ca ions during the subsequent formation of HA on the surface of the powders. The decrease in the phosphorus concentration was attributed to the formation of both amorphous calcium

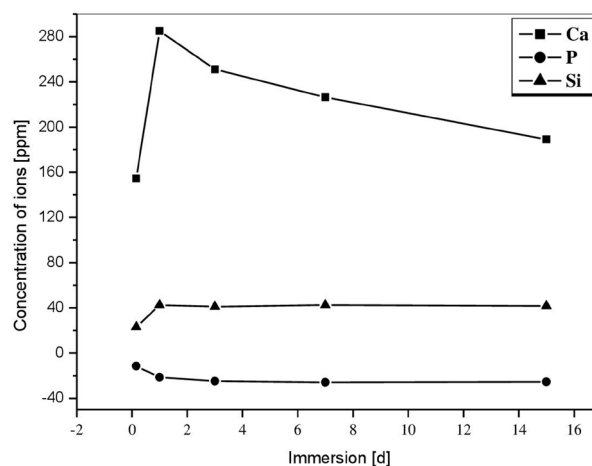


Figure 4. Changes in the Ca, P and Si concentrations of the SBF solutions after soaking the β - Ca_2SiO_4 powders for various periods.

phosphate and crystalline apatite on the surface of the powders by consuming the P ions from the SBF solutions. The ICP measurement results suggest that the Ca and Si concentrations increased while the P concentration decreased due to the formation of HCA on the surface of the β - Ca_2SiO_4 powders during soaking in the SBF solution [50].

CONCLUSIONS

β -Ca₂SiO₄ was fabricated using a solid-state reaction. The results obtained indicated that the β -Ca₂SiO₄ prepared from the local raw materials (CaCO₃) have higher reactivity in the SBF solution by showing strong hydration when soaked in it, and the hydration was favourable for formation of HCA on the surface of the powders. Furthermore, HCA was formed on the surface of powders soaked in the SBF solution for 1 day. With longer soaking periods (3, 7 and 15 days), the surface of the powders was covered by a dense and continuous HCA layer. Therefore, β -Ca₂SiO₄ ceramics should have the potential for good bioactivity and could rapidly induce HAp formation after soaking in SBF and, thus, may be used as candidate of bone repair biomaterials.

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REFERENCES

- Ducheyne P., Mauck R. L., Smith D. H. (2012): Biomaterials in the repair of sports injuries. *Nature Materials*, 11(8), 652–654. doi: 10.1038/nmat3392
- Hench L. L., J. M. Polak. (2002): *Third-generation biomedical materials Science*, 295(5557), 1014–1017. doi: 10.1126/science.1067404
- Hench L., Anderson O. (1993): Bioactive glass. In: *An Introduction to Bioceramics*, ed. L.L. Hench and J. Wilson, World Scientific, USA, p. 41.
- Hench L.L., Splinter R.J., Allen W.C., Greenlee T.K. (1971): Bonding mechanisms at the interface of ceramic prosthetic materials. *Journal of Biomedical Materials Research Symp*, 36, 117. doi: 10.1002/jbm.820050611
- Orjan H.A., Kaj H.K. (1991): On the bioactivity of silicate glass. *Journal of Non-Crystalline Solids*, 129, 145. doi: 10.1016/0022-3093(91)90090-S
- Yan H.W., Zhang K., Blabford C.F., Francis L.F., Stein A. (2001): In vitro hydroxycarbonate apatite mineralization of CaO-SiO₂ sol-gel glasses with a three-dimensionally ordered macroporous structure. *Chemistry of Materials*, 13, 1374. doi:10.1021/CM000895E
- Branda F., Arcobello-Varlese F., Constantini A, Luciani G. (2002): Effect of the substitution of M₂O₃ (M=La, Y, In, Ga, Al) for CaO on the bioactivity of 2,5CaO.2SiO₂ glass. *Biomaterials*, 23, 711. doi: 10.1016/s0142-9612(01)00173-9
- Izquierdo-Barba I., Sanlinas A.J., Vallet-Regi M. (1999): In vitro calcium phosphate formation on sol-gel glasses of the CaO-SiO₂ system. *Journal of Biomedical Materials Res*, 47, 243. doi:10.1002/(SICI)1097-4636(199911)47:23.0.CO;2-S
- Kokubo T. (1993). A/W Glass-ceramics: Processing and properties. In: *An Introduction to Bioceramics*, ed. L.L. Hench and J. Wilson, World Scientific, USA, pp.75.
- Ono K., Yamamuro T., Nakamura T., Kakutani Y, Kitsugi T. (1988): Apatite-Wollastonite containing glass-ceramic-fibrin mixture as a bone defect filler. *Journal of Biomedical Materials Research*, 22, 869. doi: 10.1002/jbm.820221004
- Kokubo T. (1990): Surface chemistry of bioactive glass-ceramics. *Journal of Non-Crystalline Solids*, 120, 138. doi: 10.1016/0022-3093(90)90199-V
- Kokubo T., Kushitani H., Saka S., Kitsugi T., Kitsugi T., Yamamuro T. (1990): Solutions able to reproduce in vivo surface structure changes in bioactive glass-ceramic A-W. *Journal of Biomedical Material Research*, 24, 721. doi: 10.1002/jbm.820240607.
- Cho S. B., Miyaji F., Kokubo T., Nakamura T. (1997): Induction of bioactivity of a non-bioactive glass-ceramic by a chemical treatment. *Biomaterials*, 18, 1479. doi: 10.1016/s0142-9612(97)00084-7.
- Lamy D., Pierrc A., Heimann R.B. (1996): Hydroxyapatite coatings with a bond coating of biomedical implants by plasma projection. *Journal of Materials. Research*, 11, 680. doi:10.1557/JMR.1996.0082
- Liu X.Y., Tao S.Y., Ding C.X. (2002): Bioactivity of plasma sprayed dicalcium silicate coatings. *Biomaterials*, 23, 963. doi: 10.1016/s0142-9612(01)00210-1
- Smith D.K., Majumdar A.J., Ordway F. (1961): Re-examination of the polymorphism of dicalcium silicate. *Journal of the American Ceramic Society*, 44(8), 405-411. doi:10.1111/j.1151-2916.1961.tb15472.x
- Cancedda R., Giannoni P., Mastrogiacomio M. (2007): A tissue engineering approach to bone repair in large animal models and in clinical practice. *Biomaterials*, 28(29), 4240–4250. doi: 10.1016/j.biomaterials.2007.06.023
- Xu S., Lin K., Wang Z., Chang J., Wang L., Lu J., et al. (2008): Reconstruction of calvarial defect of rabbits using porous calcium silicate bioactive ceramics. *Biomaterials*, 29(17), 2588–2596. doi: 10.1016/j.biomaterials.2008.03.013
- Hoppe A., Jokic B., Janackovic D., Fey T., Greil P., Romeis S., et al. (2014): Cobalt-releasing 1393 bioactive glass-derived scaffolds for bone tissue engineering applications. *ACS Applied Materials and Interfaces*, 6(4), 2865–2877. doi: 10.1021/am405354y
- Wu C., Chen Z., Yi D., Chang J., Xiao Y. (2014): Multidirectional effects of Sr-, Mg-, and Si-containing bioceramic coatings with high bonding strength on inflammation, osteoclastogenesis, and osteogenesis. *ACS Appl. Mater. Interfaces*, 6(6), 4264–4276. doi: 10.1021/am4060035
- Zhou R., Wei D., Cheng S., Feng W., Du Q., Yang H., et al. (2014): Structure, MC3T3-E1 cell response, and osseointegration of macroporous titanium implants covered by a bioactive microarc oxidation coating with microporous structure. *ACS Applied Materials and Interfaces*, 6(7), 4797–4811. doi: 10.1021/am405680d
- Martinez-Vazquez F. J., Cabanas M. V., Paris J. L., Lozano D., Vallet-Regi M. (2015): Fabrication of novel Si-doped hydroxyapatite/gelatine scaffolds by rapid prototyping for drug delivery and bone regeneration. *Acta Biomaterialia*, 15, 200–209. doi: 10.1016/j.actbio.2014.12.021
- Shi M., Zhou Y., Shao J., Chen Z., Song B., Chang J., et al. (2015): Stimulation of osteogenesis and angiogenesis of hBMSCs by delivering Si ions and functional drug from mesoporous silica nanospheres. *Acta Biomaterialia*, 21, 178–189. doi:10.1016/j.actbio.2015.04.019
- Zhao W., Wang J., Zhai W., Wang Z., Chang J. (2005): The selfsetting properties and in vitro bioactivity of tricalcium silicate. *Biomaterials*, 26(31), 6113–6121. doi: 10.1016/j.biomaterials.2005.04.025
- Correa D., Almirall A., Garcia-Carrodegua R., dos Santos L. A., de Aza A. H., Parra J., et al. (2014): Beta-dicalcium

- silicate-based cement: synthesis, characterization and in vitro bioactivity and biocompatibility studies. *Journal of Biomedical Materials Research. Part A*, 102(10), 3693–3703. doi: 10.1002/jbm.a.35041
26. Yang X., Liu M., Zhao Y., Jia H., Xu S., Li X., et al. (2014): Rational design and fabrication of a b-dicalcium silicate-based multifunctional cement with potential for root canal filling treatment. *Journal of Materials Chemistry B*, 2(24), 3830. doi: 10.1039/C4TB00129J
27. Eid A. A., Hussein K. A., Niu L. N., Li G. H., Watanabe I., Al-Shabrawey M., et al. (2014): Effects of tricalcium silicate cements on osteogenic differentiation of human bone marrow-derived mesenchymal stem cells in vitro. *Acta Biomaterialia*, 10(7), 3327–3334. doi: 10.1016/j.actbio.2014.04.006
28. Lin M., Zhang L., Wang J. C., Chen X. Y., Yang X. Y., Cui W. G., et al. (2014): Novel highly bioactive and biodegradable gypsum/calcium silicate composite bone cements: from physicochemical characteristics to in vivo aspects, *Journal of Materials Chemistry B*, 2(14), 2030–2038. doi:10.1039/C3TB21786H
29. Ohtsuki C., Kokubo T., Yamamuro T. (1992): Mechanism of apatite formation on CaO-SiO₂-P₂O₅ glasses in a simulated body fluid. *Journal of Non-Crystalline Solids*, 143, 84-92. doi:10.1016/S0022-3093(05)80556-3
30. Li P., Zhang F. (1990): The electrochemistry of a glass surface and its application to bioactive glass in solution. *Journal of Non-Crystalline Solids*, 119, 112-118. doi: 10.1016/0022-3093(90)90247-J
31. Liu X., Morra M., Carpi A., Li B. (2008): Bioactive calcium silicate ceramics and coatings. *Biomedicine & Pharmacotherapy*, 62, 526e529. doi:10.1016/j.biopha.2008.07.051
32. Mezahi F.Z., Oudadesse H., Harabi A., Lucas-Girot A., Le Gal Y., Chahar H., Cathelineau G. (2009): Dissolution kinetic and structural behaviour of natural hydroxyapatite vs. thermal treatment. *Journal of Thermal Analysis and Calorimetry*, 95, 21-29. doi: 10.1007/s10973-008-9065-4J
33. Mezahi F.Z., Oudadesse H., Harabi A., Lucas-Girot A. (2012): Effect of ZrO₂, TiO₂ and Al₂O₃ additions on process and kinetics of bonelike apatite formation on sintered natural hydroxyapatite surfaces. *International Journal of Applied Ceramic Technology*, 9(3), 529-540. doi:10.1111/j.1744-7402.2011.02742.x
34. Harabi A., Chehlatt S. (2013): Preparation process of a highly resistant wollastonite bioceramics using local raw materials: Effect of B₂O₃ additions on sintering and mechanical properties. *Journal of Thermal Analysis and Calorimetry*, 111, 203-211. doi:10.1007/s10973-012-2242-5
35. Harabi A., Zouai S. (2014): A new and economic approach to synthesize and fabricate bioactive diopside ceramics using a modified domestic microwave oven. Part 1: Study of sintering and bioactivity, accepted for publication in. *International Journal of Applied Ceramic Technology*, 11, 31-46. doi: 10.1111/ijac.12047
36. Harabi A., Belamri D., Karboua N., Mezahi F.Z. (2011): Sintering of bioceramics using a modified domestic microwave oven: Natural hydroxyapatite sintering. *Journal of Thermal Analysis and Calorimetry*, 104, 283-289. doi: 10.1007/s10973-010-1115-z
37. Ming W., Ruzhong Z., Weiqing M., Yi L. (2011): Sol-gel derived CaO-SiO₂-B₂O₃ glass/CaSiO₃ ceramic composites: processing and electrical properties. *Journal of Materials Science: Mater. Electron*, 22, 843-48. doi 10.1007/s10854-010-0223-7
38. Cho S.B., Nakanishi K., Kokubo T., Soga N. (1995): Dependence of apatite formation on silica gel on its structure: effect of heat treatment. *Journal of the American Ceramic Society*, 78, 1769-1774. doi: 10.1111/j.1151-2916.1995.tb08887.x
39. Zhong H., Wang L., Fan Y., He L., Lin K., Jiang W., Chang J., Chen L. (2011): Mechanical properties and bioactivity of β-Ca₂SiO₄ ceramics synthesized by spark plasma sintering. *Ceramics International*, 37, 2459–2465. doi:10.1016/j.ceramint.2011.03.037
40. Atalay S., Adiguzel H.I., Atalay F. (2001): Infrared absorption study of Fe₂O₃-CaO-SiO₂ glass ceramics. *Materials Science and Engineering. A*, 304-306, 796-799. doi: 10.1016/S0921-5093(00)01572-0
41. Branda F., Fresa R., Coctantini A., Buri A. (1996): Bioactivity of 1.25 CaO· SiO₂ glass: an FTIR and X-ray study on powdered samples. *Biomaterials*, 17, 2247-2251. doi: 10.1016/0142-9612(95)00328-2
42. Radin S.R., Ducheyne P. (1992): Plasma spraying induced changes of calcium phosphate ceramic characteristics and the effect on in vitro stability. *Journal of Materials Science: Materials in Medicine*, 3, 33-42. doi: 10.1007/BF00702942
43. Fowler B.O. (1974): Vibrational assignments for calcium, strontium, and barium hydroxyapatites utilizing isotopic substitution. *Inorganic Chemistry*, 13, 194. doi: 10.1021/ic50131a039
44. Ravaglioli A., Krajewski A., Biasini V., Martinetti R., Mangano C., Venini G. (1992): Interface between hydroxyapatite and mandibular human bone tissue. *Biomaterials*, 13, 162-167. doi: 10.1016/0142-9612(92)90065-v
45. Luklinska Z.B., Bonfield W. (1997): Morphology and ultrastructure of the interface between hydroxyapatite-polyhydroxybutyrate composite implant and bone. *Journal of Materials Science: Materials in Medicine*, 8, 379-383. doi: 10.1023/a:1018589018205
46. De Aza P.N., Luklinska Z.B., Anseau M.R., Guitian F., De Aza S. (2001): Transmission electron microscopy of the interface between bone and pseudowollastonite implant. *Journal of Microscopy Oxford*, 201, 33-43. doi: 10.1046/j.1365-2818.2001.00779.x
47. Orefice R., Clark A., West J., Brennan A., Hench L. (2007): Processing, properties, and in vitro bioactivity of polysulfone-bioactive glass composites. *Journal of Biomedical Materials Research- Part A*, 80(3), 565-580. doi: 10.1002/jbm.a.30948
48. Peitl O., Dutra Z. E., Hench L.L. (2001): Highly bioactive P₂O₅-Na₂O-CaO-SiO₂ glass-ceramics. *Journal of Non-Crystalline Solids*, 292(1-3), 115-126. doi:10.1016/S0022-3093(01)00822-5
49. Liu X., Ding C., Chu P.K. (2004): Mechanism of apatite formation on wollastonite coatings in simulated body fluids, *Biomaterials*, 25(10), 1755-1761. doi: 10.1016/j.biomaterials.2003.08.024
50. Z. Gou, Ding, Chang J. (2004): Synthesis and in vitro bioactivity of dicalcium silicate powders. *Journal of the European Ceramic Society*, 24, 93-99. doi:10.1016/S0955-2219(03)00320-0