

The Influence of Titanium on Physicochemical Properties of Ti-modified Hydroxyapatite Materials

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Abstract

The current and very popular approach to improving physicochemical and biological properties of hydroxyapatite implants is to incorporate some ions to HA structure. In this study we report the influence of titanium additives on sinterability, phase composition, microstructure, flexural strength and chemical stability of Ti-modified hydroxyapatite ceramics. Hydroxyapatite powders doped with various concentrations of Ti (0.5, 1.0 and 2.0 wt%) were produced by a wet method. In such a synthesis CaO, H₃PO₄ and TiCl₃ were applied as reactants. The modification of HA structure by incorporation of titanium caused thermal decomposition of hydroxyapatite. During heat treatment perovskite (CaTiO₃) and α -TCP as the secondary phases were formed. Titanium additives decreased sinterability and flexural strength of Ti-HA ceramics. The obtained materials were chemically stable (pH values measured in SBF were close to the physiological value - during the 90 days of incubation changed in the narrow range from 7.48 to 7.70). Ionic conductivity of aqueous extracts was low (5–45 μ S) and increased with the amount of titanium additive. The bioactive potential of Ti modified HA-ceramics was confirmed.

Keywords: Titanium modified hydroxyapatite, Physicochemical properties, Cell culture

WPLYW TYTANU NA WŁAŚCIWOŚCI FIZYKOCHEMICZNE MATERIAŁÓW HYDROKSYAPATYTOWYCH MODYFIKOWANYCH TYTANEM

W celu poprawy parametrów fizykochemicznych oraz biologicznych implantów hydroksyapatytowych stosuje się modyfikacje tego związku na drodze wprowadzania do jego struktury różnorodnych jonów. W niniejszych badaniach przedstawiony został wpływ dodatków tytanu na spiekalność, skład fazowy, mikrostrukturę, wytrzymałość na zginanie oraz stabilność chemiczną ceramiki hydroksyapatytowej modyfikowanej tytanem. Proszki hydroksyapatytowe o różnej zawartości Ti (0,5, 1,0 and 2,0 % mas.) zsyntetyzowane zostały przy pomocy metody mokrej. Substratami w trakcie syntezy były następujące związki chemiczne: CaO, H₃PO₄ oraz TiCl₃. Modyfikacja struktury hydroksyapatytu poprzez wprowadzenie jonów tytanowych stała się przyczyną dekompozycji temperaturowej związku. Podczas ogrzewania oprócz HA pojawiły się nowe fazy: perowskit (CaTiO₃) oraz α -TCP. Dodatek tytanu spowodował obniżenie spiekalności oraz wytrzymałości mechanicznej ceramiki Ti-HA. Otrzymane materiały charakteryzowały się stabilnością chemiczną (wartości pH mierzone podczas inkubacji próbek w SBF były zbliżone do pH fizjologicznego - podczas 90 dni inkubacji zmiana pH nastąpiła jedynie w wąskim zakresie 7,48–7,70). Przewodnictwo jonowe ekstraktów wodnych utrzymywało się na niskim poziomie (5–45 μ S) i rosło wraz ze wzrostem ilości wprowadzonego tytanu. Potwierdzono potencjał bioaktywny ceramiki HA modyfikowanej tytanem.

Słowa kluczowe: hydroksyapatyt modyfikowany tytanem, właściwości fizykochemiczne, hodowla komórkowa

1. Introduction

Biomaterials engineering is an interdisciplinary field, closely related to tissue and genetic engineering. Experimental methods and development of new materials are - apart from cell cultures and manipulations of genes - important areas of reconstructive and regenerative medicine. Longer human life as well as progress in science and medicine lead to the search for new methods in the field of biomaterials and implantology. At present, new implant materials have to not only replace the damage organs and tissue structures, but also stimulate their reconstruction [1]. In the recent years, the concepts of regenerative medicine and tissue engineering focused on stimulating the system to reconstruct the tissue

are being developed. In order to design new implant biomaterials it is necessary to define their applicational properties. Biochemical, biomechanical as well as bioelectrical aspects deciding on proper implant functioning and influencing the immunological and metabolic processes [1, 2] taking place in the system after implantation should be considered. Currently, there is a constant search for the materials that would exhibit a whole set of properties necessary to be simultaneously completely biocompatible and biofunctional. Research work aimed at improving mechanical properties and bioactivity of implant materials is performed all the time.

Ceramics based on calcium phosphates, mainly hydroxyapatite (HA) and tricalcium phosphate (TCP), give rise to particular scientific interest. Investigations on preparation

and modification of biphasic materials, *i.e.* BCP (*Biphasic Calcium Phosphate*), composed of HA and β TCP as well as on gradient materials, *i.e.* FGM (*Functionally Gradient Materials*) are also carried out [1, 3, 4].

Calcium phosphates (CaPs) – based ceramics meet a number of requirements for bone substitutes. Due to the similarity in chemical and mineral composition to the natural bone, HA shows high biocompatibility and bioactivity. Clinical investigations indicate also that hydroxyapatite is an osteoconductive material [1, 3-6]. Owing to the possibility to control porosity, the growth of living tissue into the CaPs implants and thus morphological fixation and direct bonding of the bioactive implant and the bone is possible. Not sufficient mechanical parameters, which limit the range of their applications, are a disadvantage of CaPs ceramics [1, 5]. Low values of K_{Ic} and Weibull's modulus as well as still too high Young's modulus and tendency to slow development of cracks are the reasons for which these materials can be applied only in the sites not carrying high mechanical loads [1].

Isomorphous substitutions of the cationic and anionic sublattices in the compound structure are possible. Calcium ions can be replaced by monovalent ions: Na^+ , K^+ , Li^+ , bivalent ions: Mg^{2+} , Cd^{2+} , Pb^{2+} , Mn^{2+} , Zn^{2+} as well as tri- and tetravalent ones: Al^{3+} , Cr^{3+} , Fe^{3+} , Bi^{3+} , La^{3+} , Y^{3+} , In^{3+} , Ti^{4+} . Phosphate groups PO_4^{3-} can be substituted with: CO_3^{2-} , SO_3^{2-} , VO_4^{3-} , whereas OH^- – with fluoride and chloride ions. Such substitutions result in the changes in lattice parameters and thus in stoichiometry, chemical and thermal stability of the compound [1, 5, 9 - 11]. Not many studies can be found in the literature on the effect of Ti ions on the structure of HA and its structural stability during processing at high temperatures [3, 4, 12, 13].

The aim of our studies was to obtain titanium doped hydroxyapatite and evaluation of physicochemical and biological properties of Ti-HA ceramics.

2. Materials and methods

2.1. Materials

The investigated materials: undoped HA (as reference) and HA with 0.5, 1.0 and 2.0 wt% of titanium (Ti-HA) were obtained by the wet method using chemical grade reactants: CaO (POCH, Poland), 85 % solution of H_3PO_4 (POCH, Poland) and 15 % solution of $TiCl_3$ in 10 % HCl (MERCK, Germany). The unmodified and Ti modified HA powders were ground to the size below 0.06 mm, sieved and uniaxially pressed under the pressure of 100 MPa to discs of 12 mm in diameter and 5 mm in height or rectangular samples in dimensions 40 mm \times 8 mm \times 4.5 mm. The samples were sintered in air atmosphere for 2 h at 1250°C.

2.2. Sinterability

Sintering degree of the investigated materials was determined basing on the measurements of apparent density, open and total porosities as well as shrinkage of the samples. The apparent density and open porosity of the materials were measured by Archimedes method.

2.3. Phase composition

Phase composition of initial powders and sintered Ti-HA samples was examined by X-ray diffraction (Diffractometer Bruker D8 Advance with Super Speed LynxEye detector and Philips device with X'Celerator detector). Phase quantification was made by the Rietveld method.

2.4. Mechanical strength

Flexural strength was measured on rectangular samples in the three-point bending test using Instron 3345 (head 5 kN, span 20 mm, speed 2 mm/min).

2.5. Microstructure

The microstructure of the surface before and after incubation in SBF solution and distilled water was examined by SEM (Hitachi S-4700 and Nova NanoSem 200). Chemical compositions in microareas were determined by EDS (Model 4457F_ISUS-SN and EDS with detector SUTW-Sapphire, Thermo Electron Corporation Co.).

2.6. Chemical stability and *in vitro* degradation

Chemical stability and *in vitro* degradation were evaluated on the basis of pH and ionic conductivity measurements. The studies were carried out in the SBF and aqueous solutions after incubation of the Ti-HA and HA samples. The samples were soaked during 12 weeks at the temperature 37°C (incubator Pol-Eko - ST1). Two times a week pH and conductivity were determined by Hanna H1 98129 Combo apparatus.

2.7. Biological study

Samples (6 for each type of material) were placed in the wells of a 96-well culture plate. Next, the MG-63 cells were seeded on their surfaces and cultured under standard conditions. The cells seeded on the standard tissue cultures polystyrene surface served as a positive control. After 7 days of culture the cell number (DNA content – PicoGreen test) and viability (XTT test) was determined. During the study (at the 1, 3 and 7 day of a culture) cell morphology on the investigated materials was also examined using a fluorescent microscope (Nikon Eclipse TE 200-U).

3. Results and discussion

3.1. Sintering behaviour and phase composition

Shrinkage of the sintered materials containing 0.5 and 1.0 wt% of Ti was close to that of the unmodified HA bioceramics and it was equal to ~24.8 %. The material with 2 wt% of Ti additive showed the lowest shrinkage (23.1 %) and the lowest apparent density (2.67 g/cm³). Additionally, the increase in open porosity observed starting from 1.0 wt% of Ti content (Table 1) proves worse sinterability of the Ti-doped materials.

Higher porosity of Ti-HA ceramics makes it possible to expect their lower mechanical strength on the one hand and

Table 1. Sintering degree of the materials studied.

Material	Shrinkage [%]	Apparent density [g/cm ³]	Open porosity [%]	Total porosity [%]
HA	24.3 ± 0.1	2.89 ± 0.02	4.1 ± 0.9	7.9 ± 0.6
HA - 0.5 wt % Ti	24.8 ± 0.1	2.87 ± 0.01	4.0 ± 0.7	7.5 ± 0.2
HA - 1.0 wt % Ti	24.9 ± 0.1	2.80 ± 0.01	8.7 ± 0.4	9.4 ± 0.4
HA - 2.0 wt % Ti	23.1 ± 0.1	2.67 ± 0.01	8.7 ± 0.2	10.3 ± 0.1

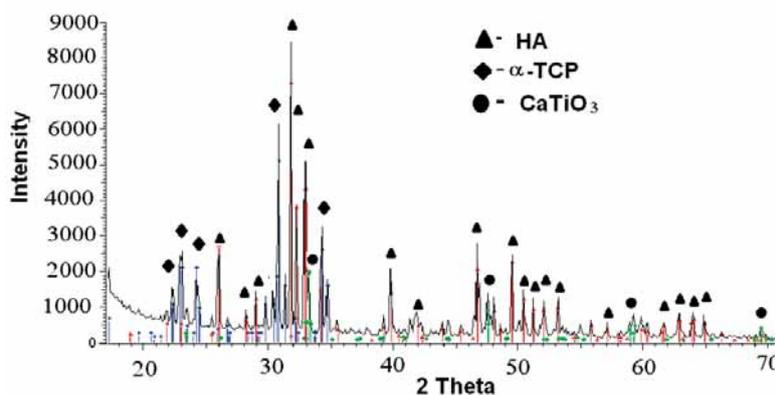


Fig. 1. XRD pattern of the HA-2.0 wt% Ti material sintered at 1250°C.

higher ability to biodegradation on the other. Hence, better osteointegration of Ti-HA ceramics in a living body can be expected.

XRD analysis of the powders calcined at 800°C showed the presence of hydroxyapatite as the only crystalline phase. However, sintering at 1250°C, apart from hydroxyapatite, resulted also in the formation of α TCP and perovskite (CaTiO_3) phases (Fig. 1).

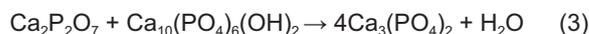
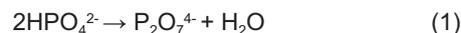
Phase composition of the materials studied is presented in Table 2. The observed thermal decomposition of Ti-doped hydroxyapatite leading to TCP and perovskite formation is in agreement with the literature data [3, 4] (α TCP was formed in our systems). The presence of harmful CaO phase, sometimes observed in such materials [3], was not detected in our investigations.

Table 2. Phase composition of the materials studied.

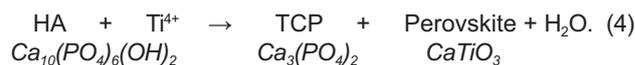
Material	Sintering temperature			
	800°C	1250°C		
	HA [%]	HA [%]	α TCP [%]	Perovskite [%]
HA	100	93.1	6.9	-
HA - 0.5 wt% Ti	100	76.1	22.9	1.1
HA - 1.0 wt% Ti	100	68.6	28.9	2.5
HA - 2.0 wt% Ti	100	44.1	49.9	5.9

In the structure of non-stoichiometric hydroxyapatite, OH^- ions form the -OH-OH-OH-OH- columnar sequence. At high temperatures, some OH^- ions escape and in this way vacancies in the structure are generated. Decrease in the strength of calcium ions bonding is also observed. As a consequence of these processes, hydroxyapatite is decomposed to a phase of calcium phosphates, more stable under these conditions [4]. Thus, a partial transformation of hydroxyapatite to TCP phase takes place. HPO_4^{2-} groups

are decomposed during heating at the temperature between 400-700°C (usually above 600°C). $\text{P}_2\text{O}_7^{4-}$ ions thus generated react with hydroxyapatite yielding TCP [1]:



At higher temperatures, in the presence of Ti^{4+} ions hydroxyapatite partially transforms to CaTiO_3 [3, 4]. The proportions of α TCP and perovskite increase as the content of titanium in the material grows (Table 2). Substitution of Ca^{2+} ions with Ti^{4+} results in destabilization and, simultaneously, creation of additional structural stresses, which favours the hydroxyapatite decomposition [4]. In the case of HA – 2.0 % Ti material this decomposition amounts to almost 50 %, whereas in the case of the undoped hydroxyapatite it is around 7 %. Such a significant difference suggests that the presence of titanium is advantageous for $\text{HA} \rightarrow \alpha$ TCP transformation. Titanium additive acts in this case as the stabilizer for α TCP phase. Reactions occurring in the systems can be schematically represented as follows [4]:

Table 3. HA and α TCP lattice parameters of the materials sintered at 1250°C.

Material	HA		α TCP		
	a [Å]	c [Å]	a [Å]	b [Å]	c [Å]
HA	9.4112	6.8797	12.8721	27.3078	12.8721
HA - 0.5 wt% Ti	9.4130	6.8817	12.8497	27.3385	12.8497
HA - 1.0 wt% Ti	9.4169	6.8803	12.8518	27.3386	12.8518
HA - 2.0 wt% Ti	9.4355	6.8787	12.8529	27.3418	12.8529

After heat treatment, HA and α TCP unit cell parameters changed. The unit cell of α TCP in the Ti-doped materials showed a lower volume than that in the undoped one (Table 3).

Most probably, the change of unit cell parameters was caused by partial substitution of Ca^{2+} cations (1.00 Å) with Ti^{4+} ions (0.61 Å), which results in a lower volume of the unit cell [4, 13]. On the other hand Wakamura *et al.* [14] suggested that Ti ions may be substituted into Ca^{2+} positions in the form of divalent ion complexes, such as $[\text{Ti}(\text{OH})_2]^{2+}$ or $[\text{Ti}(\text{HPO}_4)]^{2+}$, to provide a charge balance in HA lattice. The titanate group can be also a substitute into the location of phosphate group as $[\text{TiO}_4]^{4-}$ [15].

3.2. Mechanical strength

Results of flexural strength determination show that the material based on hydroxyapatite doped with 2 wt% of Ti exhibits significantly lower strength than pure HA and HA doped with lower amounts of Ti (Fig. 2). Lower mechanical strength of Ti-HA ceramics is related to poorer sinterability (higher porosity) and changes in the phase composition caused by the presence of titanium. Increase in the contents of α TCP and CaTiO_3 phases, showing thermal expansion coefficients different from that of hydroxyapatite, generates stresses which may result in the formation of microcracks. In the literature, the perovskite phase is sometimes treated as the contaminant whose fine grains act as inclusions which weaken the material. A synergistic effect observed in this case (increase in porosity, presence of secondary phases) leads to a significant drop in mechanical strength.

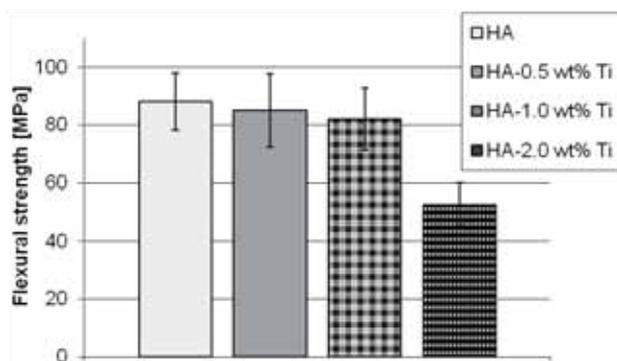


Fig. 2. Flexural strength of the materials studied.

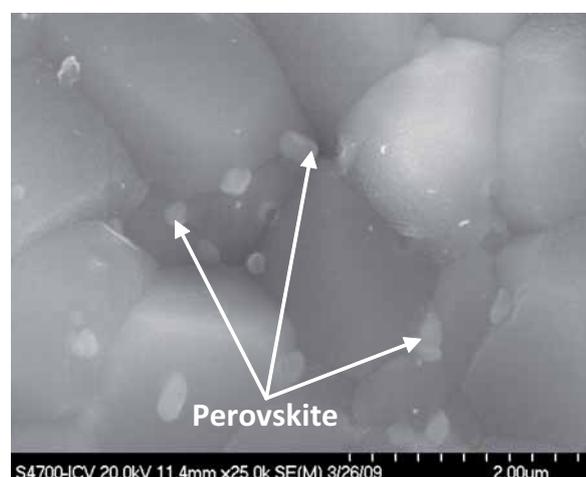
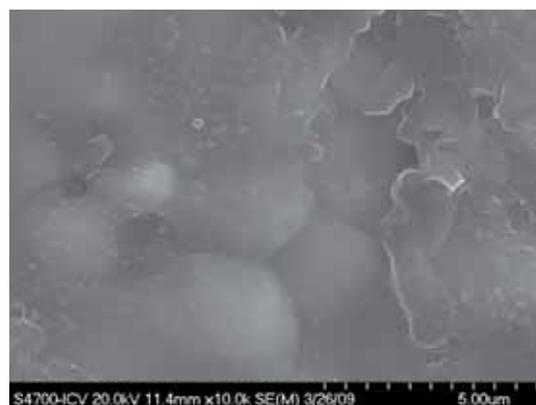
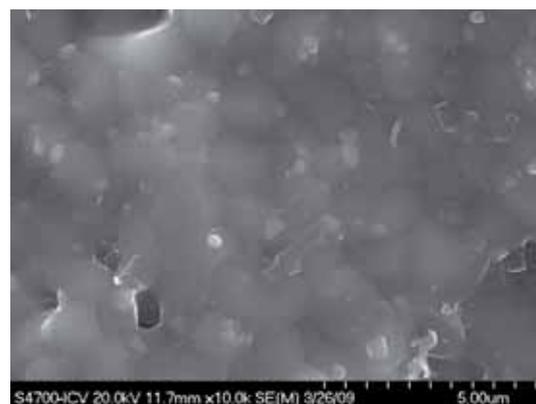


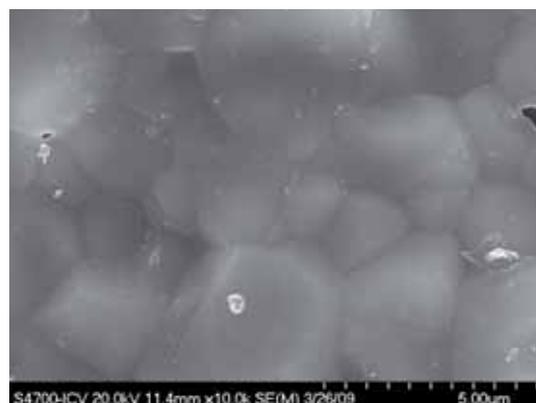
Fig. 3. SEM micrograph of HA - 2.0 wt% Ti material.



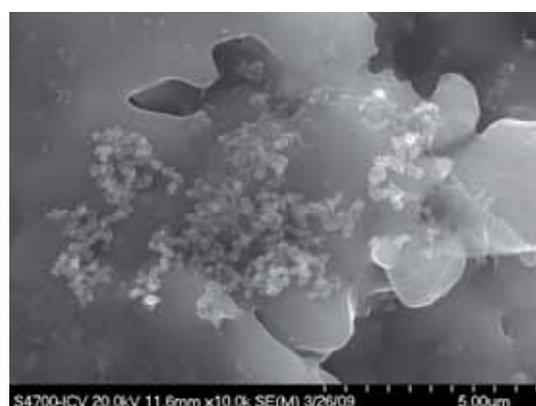
a)



b)



c)



d)

Fig. 4. SEM images of: a) HA after 4 weeks of incubation in SBF, b) HA - 2.0 wt% Ti after 4 weeks of incubation in SBF, c) HA after 4 weeks of incubation in distilled water, d) HA - 2.0 wt% Ti after 4 weeks of incubation in the distilled water.

3.3. Microstructure

SEM investigations showed that the material based on undoped hydroxyapatite contained larger grains than the Ti-HA materials. This indicates that Ti inhibits the growth of grains of hydroxyapatite ceramics. In SEM images, at the grain boundaries and on the surface of calcium phosphate grains, significantly smaller grains are visible. EDS analysis and the results of XRD investigations allowed the authors to identify them as the ones corresponding to perovskite. The size of perovskite grains increased as the amount of Ti introduced into the starting powder grew. In the HA - 2.0 wt% Ti material the grains of CaTiO_3 were relatively homogeneously distributed and their size was between 0.1 and 0.5 μm (Fig. 3).

After incubation in SBF the surface of all the materials studied was covered with the calcium phosphate coating which became thicker after a longer incubation time in SBF (Fig. 4). On the surface of HA - 2.0 wt% Ti samples soaked in distilled water, characteristic spherical apatite crystals were seen. Most probably, they were created due to intensive dissolution of $\alpha\text{-TCP}$ phase, followed by the evolution of Ca^{2+} and PO_4^{3-} ions in the form of nonstoichiometric hydroxyapatite (Fig. 4d). Such large hydroxyapatite deposits were not identified in the sinters prepared using undoped HA (Fig. 4b). Higher bioactivity potential of Ti-HA ceramics may result from their better solubility caused not only by phase composition (~50 % of $\alpha\text{-TCP}$), but also by higher porosity.

3.4. Chemical stability and in vitro degradation

Basing on pH measurements, no marked differences in chemical stability of the Ti-doped and undoped materials were found (Fig. 5). During 90 day incubation in SBF, pH of the solutions increased only slightly: from 7.48 to the maximum value of 7.70, *i.e.* close to the physiological one. The increase in pH of the solutions in which the samples were incubated can be explained by the exchange of ions between ceramics and the medium. Low crystallinity, discrepancies from stoichiometry as well as the presence of substitutions in HA and $\alpha\text{-TCP}$ lattice may lead to higher activity and solubility of the material [1]. At pH value close to physiological, Ca:P ratio becomes lower.

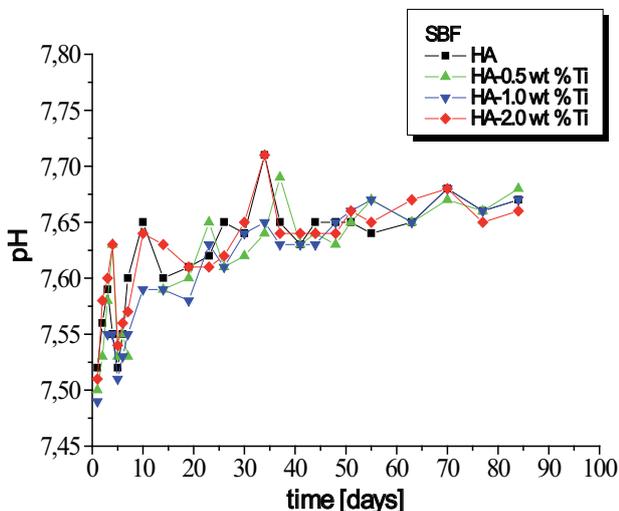
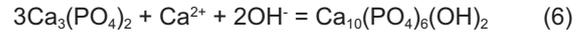


Fig. 5. Changes in pH as a function of incubation time in SBF.

The exchange of phosphate ions between the solution and the hydroxyapatite surface takes place. Hydrogen ions combine with PO_4^{3-} groups and thus HPO_4^{2-} groups are formed. They can react with the ions present in the solutions in accordance with the following equation:



The reaction of $\alpha\text{-TCP}$ phase, which is characterized by the highest solubility in the conditions close to biological, with Ca^{2+} and OH^- ions is also likely [3]. This reaction can be described by the following equation:



In order to determine the degree of degradation of the materials studied, changes in conductivity during incubation of the samples in distilled water were measured (Fig. 6). Ionic conductivity of aqueous extracts was low (5 – 45 $\mu\text{S}/\text{cm}$). The longer incubation time was accompanied by gradual increase in conductivity.

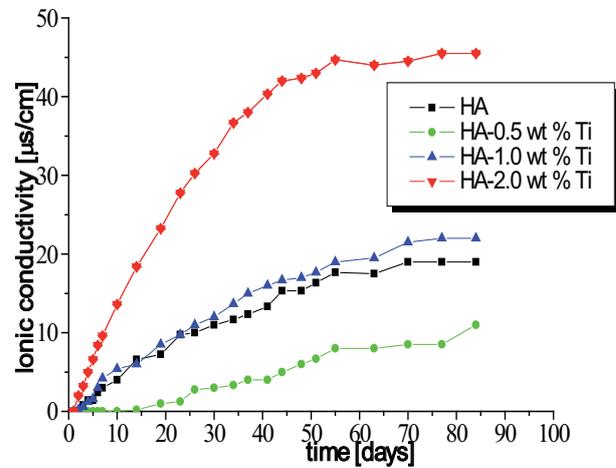


Fig. 6. Changes in conductivity as a function of incubation time in distilled water.

Growing conductivity results from the evolution of ions from the surface of the material into the surrounding medium. The rate of ions evolution is the highest for the HA - 2.0 wt% Ti material. This is most probably caused by its high open porosity which leads to higher material-medium contact area as well as by the presence of highly soluble $\alpha\text{-TCP}$ phase. After 55 days, the rate of ions evolution from the HA - 2.0 wt% Ti material stabilizes. The curve describing the dependence of conductivity on incubation time reaches plateau after ca. 50 days.

Incubation in SBF leads to the increase in weight of all the materials studied vs. time. It is connected with crystallization of apatite phases on the surface of the ceramics. The highest weight increase was detected in the case of HA - 2.0 wt% Ti:

Table 4. Weight increase [%] after various incubation times in SBF.

Material	Incubation time [weeks]			
	2	4	8	12
HA	0.5	0.46	0.79	0.79
HA- 0.5 wt % Ti	0.06	0.12	0.10	0.27
HA-1.0 wt % Ti	0.39	0.34	0.66	0.80
HA-2.0 wt % Ti	2.00	2.72	3.03	3.05

ca 3 % after 12 weeks of incubation (Table 4). This suggests high bioactivity of Ti-HA based ceramics.

3.5. Biological study

The morphology of cells cultured on the samples of HA modified with 2 wt% Ti was normal (Fig. 7) and similar to unmodified HA samples (Fig. 8). In addition, the number (DNA content) and viability (XTT test) of the cells cultured on the samples of HA modified with Ti were similar to unmodified HA samples and to the positive control (Figs. 9 and 10).

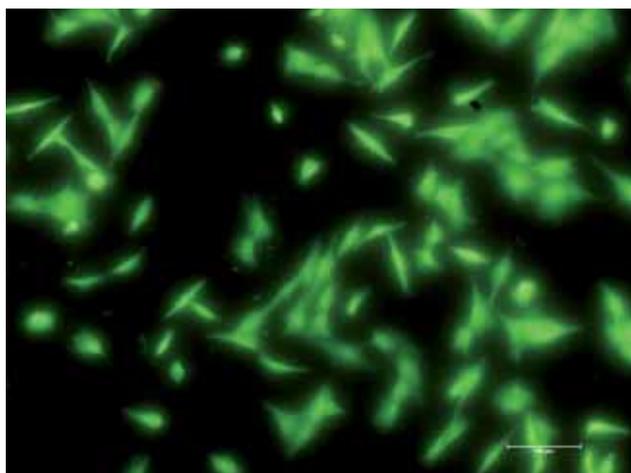


Fig. 7. Cell morphology on HA - 2.0 wt% Ti.

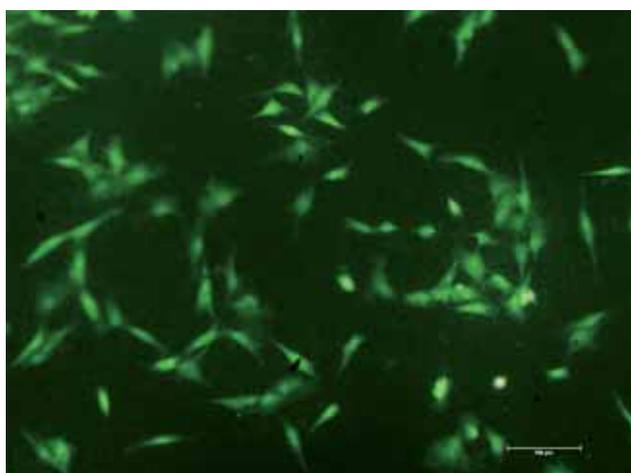


Fig. 8. Cell morphology on unmodified HA.

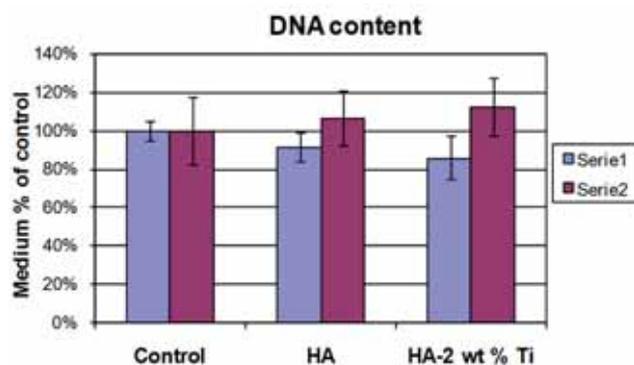


Fig. 9. DNA content.

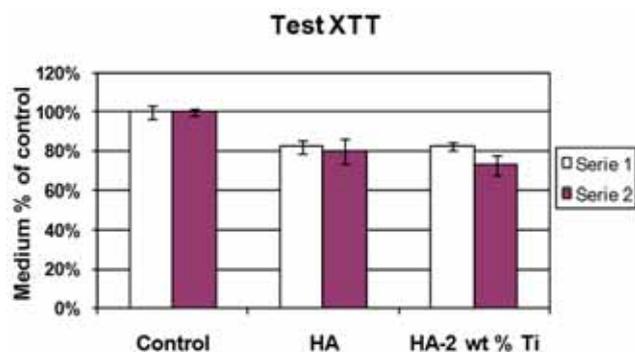


Fig. 10. Viability of cells (XTT test).

4. Summary

The influence of the titanium substitution on the structure and the thermal stability of Ti-doped hydroxyapatite was confirmed.

Most probably Ti-HA – $\text{Ca}_{10}(\text{PO}_4)_{6-x}(\text{TiO}_4)_x(\text{OH})_{2-x}$ was formed. The presence of Ti promoted the decomposition of HA phase to α -TCP and formation of perovskite (CaTiO_3) as a secondary phase.

After sintering at 1250°C three-phase ceramics: Ti-HA – α -TCP – CaTiO_3 was obtained.

Ti incorporation caused a decrease in grain size of hydroxyapatite.

Ti beyond a certain limit (1.0 wt %) decreased sinterability and promoted formation of porosity in Ti-HA ceramics.

Flexural strength of the Ti-HA material with 2.0 wt % Ti additive significantly decreased.

Ti-HA materials showed high chemical stability and for HA-2.0 wt % Ti higher ability to degradation under *in vitro* conditions with respect to the undoped HA.

Biological *in vitro* studies confirmed high biocompatibility of Ti modified hydroxyapatite.

Bioactive potential of Ti-HA containing 2.0 wt % of Ti was higher than pure HA ceramics.

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