Influence of low-temperature reaction time on morphology and phase composition of short calcium phosphate whiskers

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Abstract

The present work shows the results of the synthesis of multiphasic calcium phosphate whiskers from a mixture of biphasic calcium phosphate (β-tricalcium phosphate (β-TCP) and calcium pyrophosphate (CPP)) in the hydrogen peroxide solution-mediated process carried out in different time ranges. The process was performed at considerably lower temperature than typical hydrothermal methods used for obtaining of whiskers. Test results show that using the above-mentioned procedure triphasic calcium phosphate consisting of hydroxyapatite (HA), (β-TCP) and CPP can be obtained, where the whiskers are formed mainly from hydroxyapatite. It was found that morphology, phase composition and specific surface area of the reaction product can be controlled by changing the reaction time. The obtained triphasic HA/β-TCP/CPP short whiskers may be considered as a promising biocompatible and resorbable reinforcement in composites for bone tissue engineering with a faster resorption rate than that of HA.

Keywords: bioceramics, hydroxyapatite, low-temperature synthetic technique, whiskers

I. Introduction

Calcium-phosphate ceramics is the most popular synthetic material for bone substitution. Calcium phosphates are well tolerated by the human organism and are considered as biocompatible. They are often referred to as bioactive ceramic materials because they are soluble in biological fluids, which promotes osteoblast activity (osteogenesis) [1]. It is thought that by adsorption of bone growth-mediating proteins on the biomaterials' surface, they have the ability to create chemical bonds at the biomaterial/bone interface [2]. They are able to promote osteoconduction and osteointegration, leading to the formation of newly integrated tissue. Calcium phosphates that have received a lot of research attention include among others: hydroxyapatite (HA), α- and β-tricalcium phosphates (α-TCP, β-TCP), octacalcium phosphate (OCP) and biphasic calcium phosphates (BCP) [3]. Hydroxyapatite (HA) and β-tricalcium phos-

phate (β-TCP) [4,5] have been the most commonly studied within current research.

Synthetic hydroxyapatite has been successfully used for biomedical applications as biomaterial of choice in both dentistry and orthopaedics [6–10]. Although hydroxyapatite has favourable properties such as bioactivity and osteoconductivity and causes a rapid bone formation and strong biological fixation to bone tissues [11], it has been described as "nonresorbable" [2]. Slow resorption or lack of it, together with the structure of large and irregularly formed crystals of hydroxyapatite, makes the rebuilding of a full-blown bone impossible.

β-TCP is the material with a fast resorption rate. It undergoes a resorption process involving its partial absorption [12–14]. β-TCP is safe, non-toxic and it is used without the risk of disease transmission or immunogenic response. β-TCP is highly biocompatible and it shows an osteoconductivity higher than HA, but its mechanical strength is lower than that of HA [15].

Taking into account the mechanical properties of HA and biodegradability of β-TCP, biphasic calcium phos-

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phates (BCP) composed simultaneously of HA and β-TCP in various proportions were introduced into the range of ceramic biomaterials [16–19]. Ability to manipulate the biomaterial composition is a special advantage of BCP and it allows better control over bioactivity and biodegradation. Late or too rapid biodegradation of the implanted material may disrupt the rate of bone recovery and its structure. In general, the higher the β-TCP/HA ratio, the more biodegradable the BCP material is [20,21]. Adequate adjustment of BCP composition allows to optimize the rate of biodegradation and to improve bone repair in a specific application [22].

As the literature data show, the combination of HA and β-TCP in BCP is not necessarily the only choice for developing materials with the properties desired for reconstructive surgery. Biphasic calcium phosphate (BCP) compositions consisting of β-tricalcium phosphate (β-TCP) and calcium pyrophosphate (CPP) are also potential biodegradable ceramics for application in bone regeneration. It is known that CPP has been used as a sintering aid for β-TCP [23] and moreover as the reinforcement for composites with biopolymer [24]. The rate of calcium pyrophosphate degradation is greater than in the case of β-TCP and HA, and positive results have also been achieved when CPP alone was used [25,26]. CPP ceramics are characterized by excellent biocompatibility with bone tissue, as confirmed in *in vitro* and *in vivo* studies [27,28]. After implantation of CPP to the bone defect, direct contact between CPP and bone was observed [29], and new bone formation occurred underneath the graft [27,30,31]. CPP ceramics is bioactive, it remains easier to embed and absorbs faster than HA ceramics [32]. Grover *et al.* [33] reported that incorporating CPP into cement based on brushite stimulates bone growth *in vivo* due to the presence of alkaline phosphatase (ALP). Studies show that ALP, which is secreted by osteoblasts, hydrolyses $P_2O_7^{4-}$ released during CPP degradation and prevents the inhibition of HA formation what promotes mineralization [34]. The above information may indicate an advantage in the use of degradable CPP in regenerative medicine.

In addition, hydroxyapatite composites with polymers are also partially biodegradable [35,36]. In ceramic/polymer biocomposites, calcium phosphates are often used as fillers that improve mechanical properties of the material. It has been suggested that fibres may be the fillers that significantly enhance the strength of biomaterials based on both ceramics and polymers. Therefore, most of the researches have focused on the use of HA whiskers as promising reinforcement for biomaterials [37]. Short hydroxyapatite whiskers have been tested as reinforcing fillers among others in dental composites, bone replacements and tissue engineering scaffolds [9,38,39]. It was also found that the short calcium phosphate whiskers are non-toxic and are compatible with the human body [8]. Therefore, the recent research has also focused on strengthening weak biopolymer scaffolds with these biocompatible whiskers [40,41].

As the application of well-crystallized, stoichiometric HA whiskers in resorbable composites for bone regeneration may be limited due to its slow resorption [42], it seems to be more appropriate to use resorbable calcium phosphate powders or whiskers being bi- or triphasic mixtures. Due to that fact, it was decided to combine two resorbable calcium phosphate phases: β-TCP and CPP with HA in the form of whiskers. This combination would allow obtaining a strengthening component for composites with a controlled rate of resorption.
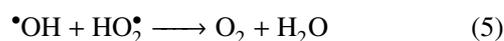
There is a number of different methods of producing calcium phosphate whiskers [43–46]. Hydrothermal homogeneous precipitation is used most frequently. Hydrothermal synthesis procedure requires special autoclaves enabling heating of aqueous solutions to high temperatures up to 200 °C [47,48]. In order to avoid problems of hydrothermal synthesis, in the present research, it was decided to use the method of obtaining HA whiskers described by Tas [44], based on H_2O_2 solution-mediated process. According to literature reports [49–51] H_2O_2 decomposes at elevated temperature and provides the higher oxygen concentration and pressure throughout the overall reaction:

$$H_2O_2 + H_2O \longrightarrow 2H_2O + \frac{1}{2}O_2 \qquad (1)$$
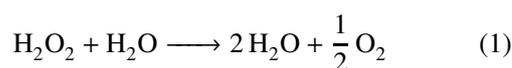
It is assumed [49] that the initial step of H_2O_2 decomposition in the liquid phase is:

$$H_2O_2 + M \longrightarrow 2\,{}^{\bullet}OH + M \qquad (2)$$

where M is a water molecule. The resulting ${}^{\bullet}OH$ radicals may further react as follows:

$${}^{\bullet}OH + H_2O_2 \longrightarrow 2HO_2^{\bullet} + H_2O \qquad (3)$$

$$HO_2^{\bullet} \longrightarrow H^+ + O_2^- \qquad (4)$$

$${}^{\bullet}OH + HO_2^{\bullet} \longrightarrow O_2 + H_2O \qquad (5)$$

and the overall stoichiometry is still given by reaction (1). The above-presented reactions occur most likely on the surface of substrate particles and it can probably improve their dissolution and formation of new calcium phosphates [44,49]. This may allow the process of calcium phosphate whiskers formation to carry out at temperatures lower than 100 °C and without the need to use special autoclave for hydrothermal synthesis.

The present paper shows the results of the synthesis of short multiphasic calcium phosphate whiskers from biphasic calcium phosphate (β-TCP/$Ca_2P_2O_7$) by the H_2O_2 solution-mediated process. The process was carried out at a temperature of 95 °C that is considerably lower than for hydrothermal methods. Formation, morphology and phase composition of the obtained whiskers were studied as a function of reaction time.

II. Experimental

2.1. Materials

The starting calcium phosphate powder (β-TCP, 96%, Product No. 21218) was supplied by Sigma-Aldrich as product of Fluka Chemie GmbH, Buchs, Switzerland and 30% solution of H_2O_2 (Catalog No. BA5193111) was supplied by Avantor Performance Materials Poland S.A., Gliwice, Poland.

Before starting the synthesis the calcium phosphate powder was analysed by XRD and characterized as biphasic calcium phosphate consisting of β-TCP (84.8%) and $Ca_2P_2O_7$ (15.2%).

2.2. Preparation of whiskers

Multiphasic calcium phosphate whiskers were synthesized by one-pot technique according to the method previously described [44], applying slightly higher temperature and different reaction times. Taking into consideration that the decomposition rate of H_2O_2 increases with the raising of temperature, it was decided to carry out the process at higher temperature than previous authors in order to achieve a higher reaction rate. However, since the decomposition of H_2O_2 in water solution provides higher oxygen concentration and pressure, due to the safety of the process only 5 °C higher temperature was applied compared to the above-mentioned procedure. In the method previously described [44] the time of reaction was 48 h and authors did not determine the dependence of products' phase composition on the reaction time. Since our substrate subjected to the reaction was the biphasic mixture of calcium phosphates, it was expected that these two phases could not react identically giving the products with different phase composition. To determine the differences and the dependence on the reaction time, the process was carried out in time range 8–96 h. The final procedure was in brief as follows: 4 grams of the starting powder were placed in 250 ml capacity Pyrex glass bottle and then 100 ml of 30% solution of H_2O_2 was added. The bottle was capped and shaken for 2 min, followed by heating the capped bottles for 8–96 h (undistisbed) in a thermal chamber at 95 °C. The obtained whiskers were finally filtered, washed four times with 500 ml of distilled water and dried overnight at 90 °C.

2.3. Characterization

Microscopic observations of the starting powder particles and the obtained whiskers were performed using a field emission scanning electron microscope (Nova NanoSEM 200, FEI). Imaging of non-sputtered samples was conducted in low vacuum conditions using vCD detector at 15 kV accelerating voltage. Non-model chemical analysis involving differences in the chemical composition between powder particles and whiskers in low vacuum conditions was performed using EDS detector, SDD Apollo X model, EDAX. However, imaging of sample microstructure was performed in high vac-

uum conditions using ETD detector at 10 kV accelerating voltage. Before the study, the samples were covered with conductive material (10 nm gold film) using a sputter coater, Leica EM SCD500.

Morphology of whiskers was also observed by a STEM detector installed in SEM. In STEM observations, the whiskers were placed on a copper mesh and observed at 25 kV accelerating voltage.

The phase composition was analysed by Bragg-Brentano X-ray diffraction method (XRD) on a Bruker-AXS D8 DAVINCI diffractometer designed for a copper anode tube. The diffractograms were recorded in an angular range from 4° to 90° 2θ (Cu Kα), measuring step 0.019° and measurement time: 2 s/step. Quantitative analysis was performed by Rietveld method using TOPAS v5 program.

Functional groups of the samples were identified by Fourier transform infrared spectroscopy (FTIR). Measurements of absorbance were made using a TENSOR 27 (BRUKER) equipped with a DLaTGS detector. The analysis was performed in the wavelength range from 400 cm^{-1} to 4000 cm^{-1}. The samples were prepared as pressed pellet shape KBr moulds.

The specific surface area of the samples was measured by BET (Brunauer, Emmett and Teller) method using nitrogen adsorption at -195.8 °C with Gemini VII instrument, Micromeritics. Before the measurements were taken the samples were degassed for 1 h at 120 °C.

III. Results and discussion

Due to the fact that the synthesis of pure β-TCP powder requires an accurate control of parameters such as pH, temperature, concentrations or initial Ca/P molar ratio, it happens that the final product does not have the right degree of purity and contains various by-products. It is known that if the initial Ca/P molar ratio is lower than 1.50, CPP will appear [52]. Furthermore, Ca-deficiency of 1 wt.% can result in the formation of about 5 wt.% CPP by-products, while, Ca-excess of 1 wt.% can result in the formation of about 10 wt.% HA by-product in the synthesis of β-TCP powders [53,54]. Hence even in commercial products of β-TCP there are often present by-products of CPP. For this reason before starting the synthesis, the β-TCP powder was analysed by XRD. The powder was characterized as biphasic calcium phosphate consisting of β-TCP and $Ca_2P_2O_7$ and such mixture of two phases was accepted as a starting material in this work.

Test results indicate that by using the above-mentioned procedure with H_2O_2 solution-mediated process short calcium phosphate whiskers were obtained. Starting material powder consists of low porosity strongly integrated agglomerates with the structure presented in Fig. 1. As it can be seen, the temperature treatment of the starting powder with H_2O_2 solution led to the formation of whiskers and aggregates of different sizes (from several to tens of μm). Figure 1 shows also
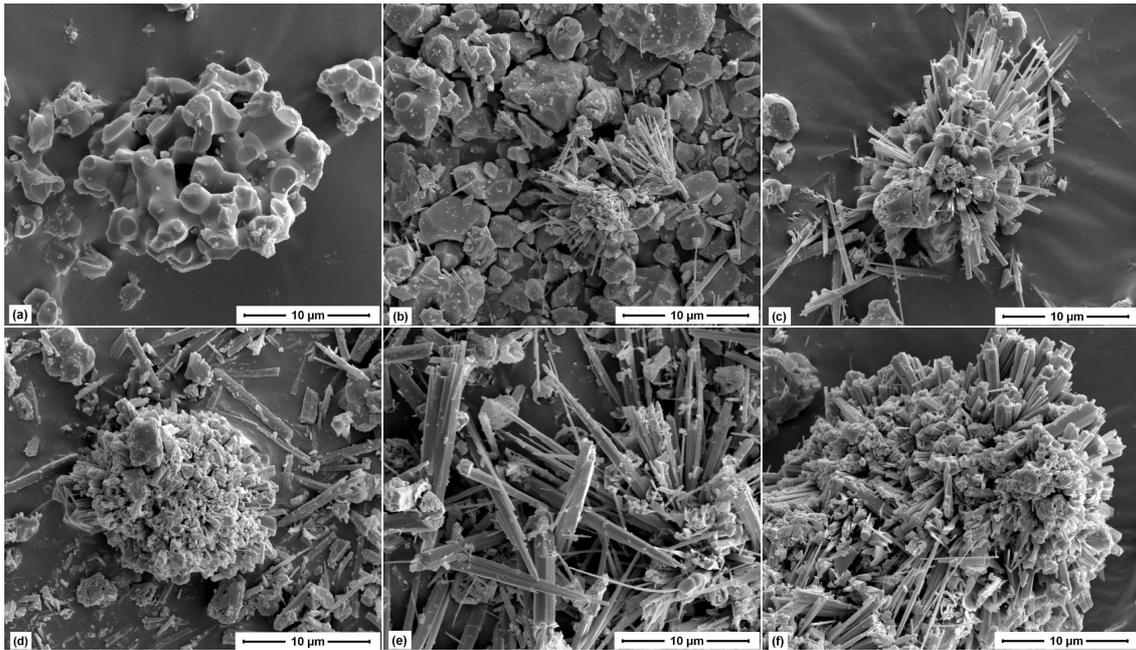
Figure 1. SEM micrographs of the starting powder (a) and whiskers obtained after the following periods of time: b) 8 h, c) 24 h, d) 32 h, e) 48 h and f) 96 h

that a very small amount of whiskers was obtained after 8 h. The whiskers appear initially as aggregations of fine needles anchored on the starting powder particles. Thus, the growth of crystals starts on the surface of these particles. The oxygen deriving from H_2O_2 decomposition was most likely adsorbed on the surface of particles nucleus, which influenced the anisotropic growth on the surface layer and promoted the spontaneous formation of rod-like agglomeration [55]. Small particles of the starting powder can be seen in the reaction products obtained after the reaction time of 32 h. An increase in reaction time led to the formation of a higher number of single whiskers and aggregates. After 48 h the starting particles were difficult to detect in SEM images. Length and width of the whiskers also increased with reaction time. The mean length of the whiskers prepared after 48 h (10–30 μm) was longer than those prepared after the shorter time and the same or just slightly shorter than those prepared after longer period. The obtained whiskers had a mean width of 1–3 μm.

The progress of the reaction is confirmed by observations of the STEM morphology (Fig. 2). The start-

ing particles in the STEM image are in the form of oval flakes and it can be seen that the boundaries of the particles are clear and the surfaces are smooth. As the reaction progresses, the surface of the substrate particle dissolves, which is shown on the STEM image as an unclear and rough border of the starting particle from which the whiskers grow.

Differences in the chemical composition between powder particles and whiskers after 48 h of reaction were determined by using a non-model chemical analysis technique based on EDS detector (Fig. 3). EDS spectra show that signals intensity for Ca and P within the whiskers area and powder particles area differ. The determined ratio of Ca/P signals intensity for whiskers area is about 1.67. The same ratio for powder particles area is about 1.5 or 1.0 depending on the selected point on the particle surface. It indicates that during reaction progress hydroxyapatite grows in the whiskers form (Ca/P = 1.67). Small powder particles present in the reaction product are unreacted residues of β-TCP (Ca/P = 1.5) and $Ca_2P_2O_7$ (Ca/P = 1.0).

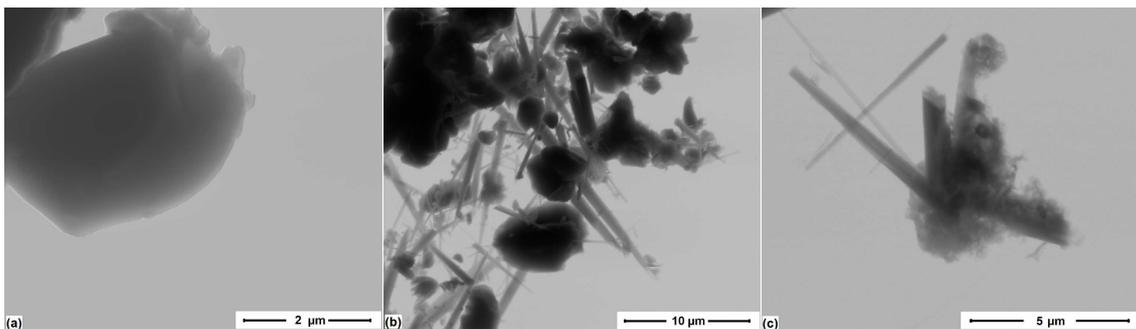The observed product's morphological changes dur-

Figure 2. STEM images of starting particles (a) and whiskers obtained after the following period of time: b) 24 h and c) 96 h
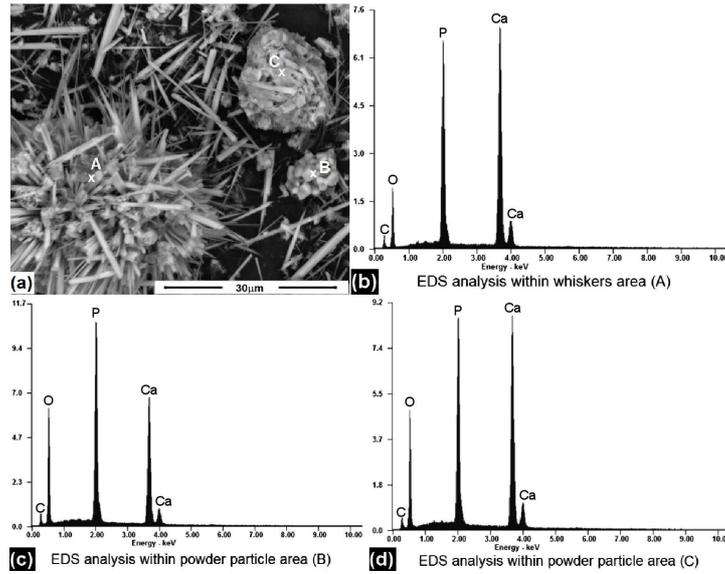
Figure 3. SEM image of the product obtained after reaction time of 48 h (a) and EDS analyses within whiskers area (b) and powder particles area (c,d)
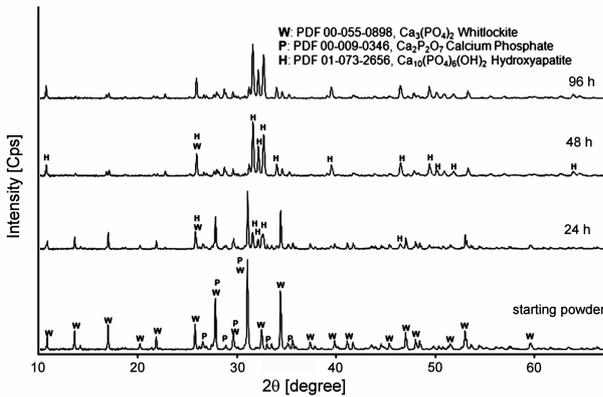

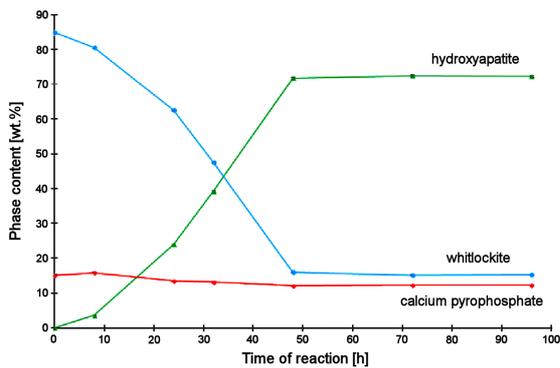**Figure 4. XRD patterns of the starting powder and samples obtained after different reaction times**

Figure 5. Phase composition of the reaction products obtained after different time of reaction carried out at 95 °C in H_2O_2 solution

ing the process are supported by XRD analysis. The XRD pattern of the starting powder (Fig. 4) shows presence of 84.8% of whitlockite ($Ca_3(PO_4)_2$) and 15.2% of calcium pyrophosphate ($Ca_2P_2O_7$). The XRD patterns of the obtained product show that calcium phosphates

were composed of three main phases: hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), calcium pyrophosphate ($Ca_2P_2O_7$) and whitlockite ($Ca_3(PO_4)_2$) (Fig. 4). The intensity of main peaks of whitlockite was decreased with reaction time. It is also noticeable that by increasing the reaction time hydroxyapatite became the dominant phase, while calcium pyrophosphate appears at an almost stable level during the process. Significant changes in the content of individual phases were observed after 48 h of synthesis. After this time, hydroxyapatite, whitlockite and calcium pyrophosphate contents were about 72%, 15% and 12%, respectively (Fig. 5).

These results are confirmed by FTIR spectra (Fig. 6). The bands at 565, 609, 1033, 1093 cm⁻¹ were attributed to the bending and stretching modes of the phosphate PO_4^{3-} groups [8]. The bands at 729 and 1215 cm⁻¹ were attributed to $P_2O_7^{4-}$ groups in calcium pyrophosphate [9,10]. Two absorption bands at 945 and 973 cm⁻¹ correspond to whitlockite [8] and their intensities decrease with the reaction progress.
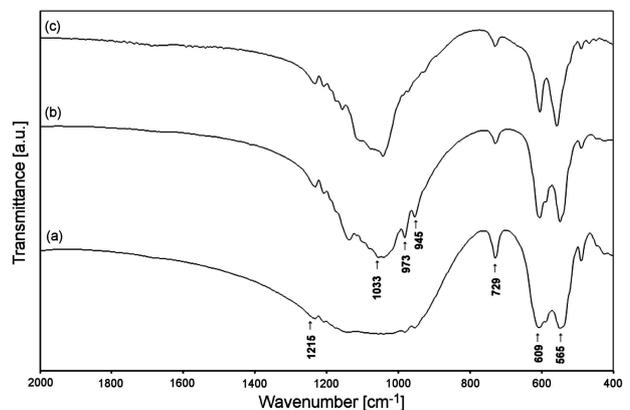

Figure 6. Fourier-transform infrared spectra of: a) starting powder and samples obtained after reaction time of: b) 8 h and c) 48 h

Phase and FTIR analyses of the obtained products completely oppose the literature's [44] where produced whiskers were found to be biphasic mixtures of apatitic calcium-phosphate and octacalcium phosphate ($Ca_8H_2(PO_4)_6 \cdot 5H_2O$). In our tests XRD patterns did not show the characteristic peak of OCP at around $2\theta = 5°$ and no characteristic FTIR absorption bands of OCP at 865 and 910 cm^{-1} were observed [56,57].

The obtained results for the samples prepared in H_2O_2 solution were compared with the results of reaction carried out under the same conditions in deionized water. According to Tas [44], the β-TCP powder did not transform to apatitic calcium-phosphate when heated in deionized water at 90 °C for 48 h. In our research it was observed that biphasic calcium phosphate (β-TCP/$Ca_2P_2O_7$) heated in deionized water at 95 °C for 48 h transformed to hydroxyapatite. However, the transformation was very slow, only to a small extent. After 48 h only 1.51% of hydroxyapatite was observed in the reaction product (Fig. 7). In order to increase the content of hydroxyapatite the time of reaction was extended to 96 h and then to 192 h. It did not show any large effect on the increase in the hydroxyapatite content, which proves that the yield of hydroxyapatite whiskers during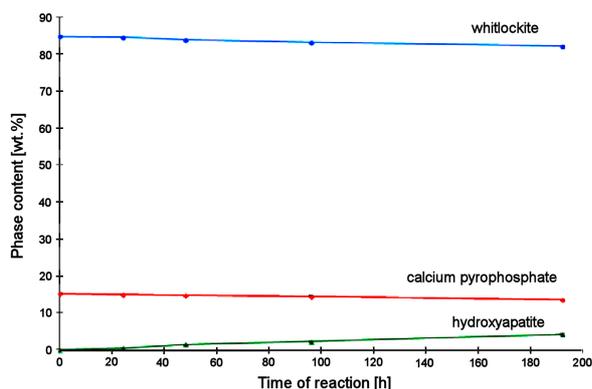 the reaction carried out at 95 °C in water is insignificant compared to the H_2O_2 solution-mediated process. Thus, in order to obtain the desired product at a suitable time, it seems to be reasonable to use H_2O_2 solution-mediated process.

Since the assumption of the work was to obtain a material with increased resorbability, it was decided to analyse the specific surface area of the products obtained in H_2O_2 solution in order to investigate the development of their surface. The results, presented in Fig. 8, indicate that the specific surface area of the obtained products increases with the increase of reaction time up to 48 h and after this time it decreases slightly. As it was observed in Fig. 2, as the reaction progresses, the smooth surface of highly integrated substrate molecules dissolves, which results in a change in its structure. It becomes rough and uneven, with numerous pores and channels, which increases the surface development despite the geometry of HA whiskers created at the same time. After some time, due to the strong growth of HA whiskers on the starting material particles, their surface can be blocked and they do not undergo further reaction. Probably because of that reason a certain amount of unreacted substrate was still present in the final product. The HA whiskers, however, are still growing for some time until the Ca and P ions are depleted from the solution. It is worth noting that 48 h is the reaction time after which, according to the results of XRD, the phase composition of the reaction product is almost stable. The specific surface area of the products obtained after 48 h indicates a good development of their surface, and this may affect the rate of resorption of the obtained material.

IV. Conclusions

Simple one-pot technique was used for synthesis triphasic calcium phosphate whiskers using biphasic β-TCP/CPP powder and 30% H_2O_2 solution. The obtained samples were characterized as a mixture of hydroxyapatite (HA), calcium pyrophosphate (CPP) and β-tricalcium phosphate (β-TCP) phases. It was observed that the phase composition of the reaction product can be controlled by the change of reaction time. The reaction time also affects morphology, size and specific surface area of the obtained products. Reaction time of 48 h is optimal as the content of HA in whiskers is about 72% and the specific surface area reaches its maximum. The triphasic HA/β-TCP/CPP short whiskers obtained in the study may be considered as a promising biocompatible and resorbable reinforcement in composites for bone tissue engineering applications with a faster rate of resorption than that of HA.

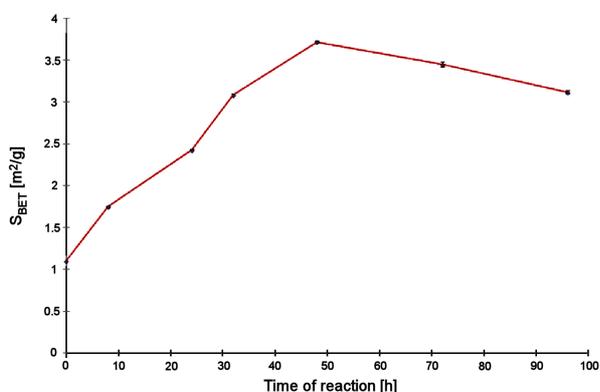**Figure 7. Phase composition of reaction products in function of time of reaction carried out at 95 °C in H_2O_2**

Figure 8. Change of specific surface area S_{BET} of reaction products with time of reaction carried out at 95 °C in H_2O_2 solution

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