

BIOACTIVITY ASSESSMENT OF CERAMIC NANOPARTICLES USED AS A FILLER IN NANO-COMPOSITE MATERIALS

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Abstract

The paper presents research on degree of bioactivity of nanometric ceramic particles used as a nanofiller in nanocomposite materials based on polymers. The nanoparticles used in our examination were: different bioceramics powders such as: hydroxyapatite (HAp), β -phosphate (V) calcium (β TCP), silica (SiO_2) and bioglass (BG). Based on ζ -potential measurements dynamics of processes occurring on the surface of nanoparticles in stimulated body fluid (SBF) was determined and it confirmed possibility of apatite formation. This study showed predominance of bioglass over other bioceramic materials, Bioglass nanoparticles were the most bioactive ones. In the end of the experiment the bioacermic particles were used as a nanofiller of poli-L/DL-lactide (PLDLA) matrix composites. The composite materials were prepared by casting from solution. Bioactivity tests were performed in simulated body fluid (artificial plasma of various ions concentration, and SBF 2SBF). Based on surface microstructure observed in the SEM (EDS) analysis were confirmed the degree of bioactivity of various materials depending on the type nanofiller.

Keywords: nanocomposite, bioactivity, scaffolds, regenerative medicine, bioceramics, zeta potential
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Introduction

Currently, the greatest interest in tissue engineering materials is focused on composites and nanocomposites based on polymers. They better match with properties of the material of living tissue. Moreover, nanoparticles characterized unique surface properties which influence on mechanical properties, physicochemical properties and durability of nanocomposites materials. Ceramic nanoparticles in polymer matrix shown similar structure which can be find in natural tissue – bone tissue. Introduction of ceramic phase can improve such characteristics of the material as bioactivity. It is guarantee better reactivity nanocomposites surface and faster fixation implant made with nanocomposites materials into human body [1,2].

Therefore the most commonly used materials are biodegradable polymers (synthetic or natural for example: PLA, PGA,) and bioactive ceramics [3]. For polymers based on lactic acid and glycolic acid (PLA, PGLA) degradation products appear to be safe because of their presence in the

metabolic pathways of the body. Unfortunately, the change of degradation degree of the polymer can lead in their case to local pH changes resulting from increased concentration of the metabolites. This may result in acceleration or inhibition of tissue regeneration [4]. Applications of bioceramic particles as the fillers provide bioactivity to the material (osteoconductivity - HAp or osteoinductivity – BG). The phenomenon of bioactivity of bone tissue applies to ability of an implant surface to the formation of chemical bonds with the bone tissue without forming an intermediate layer (connective tissue). Hydroxyapatite is similar to natural bone apatite, HAp with β tri-calcium phosphate(V) belong to osteoconductive ceramics. In case of BG (bioglass) and silica process of formation of bond between implant and bone is multistage, in which formation of silica gel is a common stage for silicon materials [3]. Calcium ions layer on the negatively charged surface of the materials is future nucleation center of hydroxyapatite. Hydroxyl groups (in the form of Si-OH, P-OH) are responsible for the negatively charged surface. Surface properties play a key role in the formation of apatite layer. In the case of bioactive materials their electrical double layer is one of the most important factors deciding whether the material is able to produce permanent connection to the bone in the form of hydroxyapatite apatite similar to bone apatite. Electrokinetic potential (ζ -potential) is used for characterization of electrical properties of the surface [5,6]. Presence of the nanofiller increases (usually faster) the degree of degradation of the polymer matrix and enhances its mechanical properties.

The paper presents the process of production of nanocomposite materials based on resorbable polymer (PLDLA) which was modified with the nanometric bioceramics particles. The research was performed in several stages. The first part concerned in the analysis of change of ζ potential of the nanoparticles in SBF suspension vs. time. In second stages nanocomposite materials which were incubated in SBF and double concentrated 2SBF for 3 and 7 days were prepared. Changes in the microstructure of the nanocomposite materials (SEM) confirmed their bioactivity.

Materials and method

As the nanofillers were used: HAp (particle size <200 nm, BET), silica (particle size 5-5nm, TEM) β -phosphate (V) calcium (β TCP) (particle size <100 nm, TEM) produced by Sigma-Aldrich. Bioglass 45S5 was obtained at AGH-UST in Krakow. The bioglass particles were characterized by submicron size (average particle size – 800 nm). All materials were prepared using synthetic copolymer of L/DL-lactide (the ratio of units L:DL 80:20, MW 20 kDa) from Purac®. The polymer had the FDA attestation confirming its biocompatibility.

Polymer-ceramic nanocomposite materials obtained using the casting from solution method. The first stage was prepared solvent mixture. As solvent used DCM and DFM (POCh, Gliwice) in a weight ratio of 9:1. Next polymer PLDLA was dissolved in solvent mixture (DCM:DMF, 10:1). The mixture of the polymer was homogenized mechanically. To the thus prepared solution was added ceramic powder in an amount equivalent to 2%wt. the filler content in the composite (after drying). After introduction of a mixture of nanoadditive used ultrasonic homogenizer. Solvent was evaporated by 24 hours on the air. All nanocomposite materials were examined in the bioactivity test – incubation in simulated body fluid, one concentrated (SBF) and double concentrated (2SBF) in in vitro condition (37°C).

The ceramics nanoparticles were incubated in SBF solution for one to ten days. The rate of change of zeta potential vs. time of the bioceramics nanoparticles in SBF suspension determined changes of their surface chemistry.

Study of electrokinetic potential (ζ) of the nanoparticles in SBF solution were performed using Nanosizer ZS (Malvern Instruments). The scanning electron microscope (SEM Nova NanoSEM 200, FEI) was used to observe surface microstructure of the nanocomposites after incubation in plasma in two time intervals i.e. 3 and 7 days. Bioactivity test was carried out by incubation of the material in the immersion medium - artificial plasma-alone (SBF) and double concentrated (2SBF) for 3 and 7 days.

Results and discussion

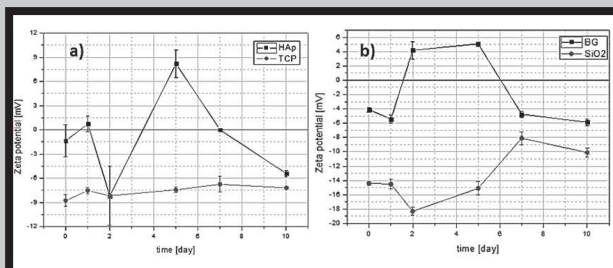


FIG.1. Zeta potential vs. time for materials from group: (on the left) CaPs-calcium phosphate, (on the right) silica.

Studies of electrokinetic potential (ζ) of the ceramic nanoparticles in SBF solution showed that the fastest changes surface charge occur in for bioglass and hydroxyapatite (FIG. 1). Bioglass particles earlier achieved potential greater than zero than HAp particles. Change of sign of the potential observed in bioglass was caused by the diffusion of the calcium ions from the environment towards negatively charged silica surface. After reaching the maximum value, the potential decreased. It is the starting point of transport of phosphate ions to the surface and crystallization of calcium phosphate on the surface. This way most stable HAp (pH>4.2) can be observed.

For the hydroxyapatite nanoparticles surface itself is the center of crystallization. OH⁻ group attracted calcium ions (positive ζ potential), which then together with phosphate ions led to precipitation of apatite (drop potential).

In the case of the silica nanoparticles noticeable changes in the ζ potential in direction the positive values were observed which indicated enhanced diffusion of Ca ions. It is possible that in this case the bioactivity is only partial and not on the entire surface or the rate of change of the potential is too high and thus difficult to observe. The β -TCP particles showed through the entire incubation process only small fluctuations of the surface charge. This phenomenon consisted of dissolution and hydrolysis of the same ceramic material.

The observations of the surface microstructure of the nanocomposites modified by ceramic nanoparocles (SEM/EDS) showed that the best bioactive potential characterized polymer with bioglass. Only PLDLA/BG had the ability of forming a layer rich in phosphorus and calcium in both types of medium immersion (SBF and 2SBF). In case of materials incubated in SBF for 7 days, on the surface of the composites of HAp, β -TCP, SiO₂ precipitates occurred which contained an increased content of Ca and P (EDS analysis). Apatite nucleation was observed locally. The first precipitates on the surface of each materials were chlorides (crystals with regular shapes), and phosphates precipitated later on the materials surface (SBF). For samples incubated in 2SBF (FIG.2) it was observed that apatite layer precipitated fastest on the edge of materials: PLA/HAp, PLA/ β TCP. The nanocomposite of silica surface apatite was covered after 14 days bioactivity test.

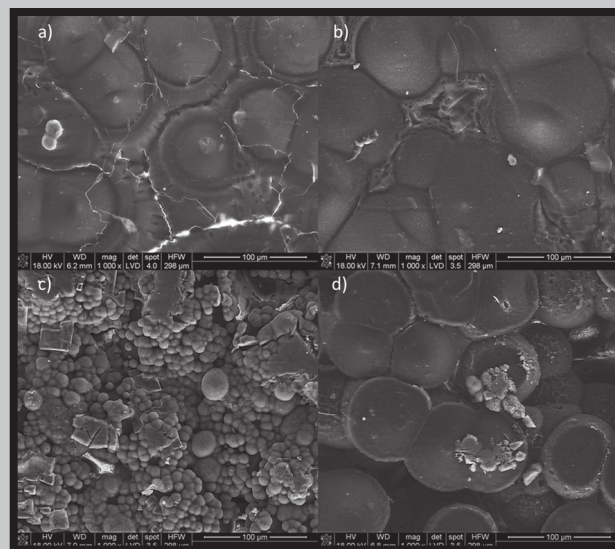


FIG.2. SEM image of a surface of PLDLA/2% a) HAp b) TCP c) BG d) SiO₂ after 7 days incubation in 2SBF.

The richest apatite layer was observed on the surface of the composite with bioglass. Almost the entire surface of the material was coated with calcium phosphate. The phosphates precipitates had the characteristic shape of "cauliflower" (FIG.2c). The relatively small amount of the precipitates in the form of calcium phosphate on the surface of the nanocomposite materials based on polylactide may be caused by several factors. The first one is the very small amount of the nano-additive (2%wt.). The second reason of moderate bioactivity of the received materials is slow degradation of PLDLA matrix covered nanoparticles.

Conclusions

Results of the zeta potential measurements showed that the most bioactive material seed to be bioglass, while β -TCP had the lowest degree of bioactivity. Analysis of the SEM images with EDS method led to following biological activity of the nanoparticles: BG>HAp> β TCP>SiO₂. The nanocomposite material with 2%wt. bioglass content in the PLDLA matrix has a good chance to find in the group of bioactive materials for scaffolds for tissue engineering.

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