Pyridoxine Incorporated in Hydroxyapatite / Polyurethane Composites

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This work presents a study on the possibility of hydroxyapatite thin layers deposition on porous polyurethane support using biomimetic method, and pyridoxine (vitamin B_6) incorporation in this layer. To achieve the formation of hydroxyapatite deposition on the surface of polyurethane and to incorporate the pyridoxine in the apatite layer, two types of biomimetic solutions, i.e. a supersaturated calcification solution (denoted SCS) and a modified SCS solution (denoted M-SCS) were used. The M-SCS solution was prepared by adding in the original SCS solution appropriate quantities of pyridoxine. The hydroxyapatite deposits are investigated by scanning electron microscopy (SEM) and Fourier-transform infrared transmission spectroscopy (FT-IR). The results obtained from SEM images and FT-IR spectra have shown that porous polyurethane/hydroxyapatite scaffolds with an interconnected network were produced, and pyridoxine is incorporated in the apatite layer.

Keywords: Hydroxyapatite, polyurethane, pyridoxine, scaffold

The nutrients such as vitamin B_6 perform a wide variety of functions in the human body and are essential for health. Vitamin B_6 is a water-soluble vitamin that exists in three major chemical forms: pyridoxine, pyridoxal, and pyridoxamine. Pyridoxine is used for many diseases such as arthritis, allergies, acne and various other skin conditions, etc. Clinical studies shown that vitamin B_6 is important in bone health, and deficiency in this vitamin leads to osteoporosis. It seem that vitamin B_6 helps break down homocysteine, a methionine metabolite, which is believed to promote osteoporosis [1,2]. Therefore, incorporation of vitamin B_6 in biomaterials that can be implanted in the body can improve their biointegration process and may act locally by its action on homocysteine.

The bioceramics based on calcium phosphates are biocompatible and bioactive because they develop links with natural bone and teeth. Between these compounds, hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) has the chemical structure of the mineral phase of bones and teeth. It is known that bones and teeth are composed of two major components, an organic and an inorganic phases, the latter being a mineral phase that contains mainly biological apatite. Hydroxyapatite can be synthesized by biomimetic methods [3], hydrothermal methods [4], sol-gel process [5], precipitation reactions [6], etc.

In the last years, many researches have been devoted to the possibility of obtaining simple or composite biomaterials that mimic natural bone tissue, to allow restoration of defects in the bones or teeth [7]. Most of these biomaterials are based on calcium phosphates, especially hydroxyapatite [8]. To be used in bone reconstruction, biomaterials must present a series of physical and chemical properties. They must be made in the form of matrices or scaffolds with three-dimensional structures exhibiting tailored porosity, pore size and interconnectivity.

From biological point of view, it is interesting to combine polymers and bioceramics to produce scaffolds for bone tissue engineering because natural bone is a composite materials based of a naturally polymer (collagen) and biological apatite.

The fabrication of porous polymer-ceramic composite scaffolds involves a number of processing techniques such as: solvent casting (with and without particle leaching), thermally induced phase separation, microsphere sintering, inorganic coating, etc. [9]. A commonly method used to obtain biocomposite materials is to coat the polymeric scaffold with a thin layer of inorganic particles. These coatings can be achieved by various methods such as: electrophoretic deposition, slurry dipping, biomimetic methods, etc. [10-13].

This work presents a study on the possibility of obtaining hydroxyapatite thin layers on porous polyurethane support using a biomimetic method. Also, our study aims to include pyridoxine in hydroxyapatite layer with its controlled release ability. To investigate hydroxyapatite coatings formation on polyurethane surface, two types of solutions such as supersaturated calcification solution (SCS) and modified SCS (M-SCS) were used. The M-SCS solution was prepared with added at original SCS solution appropriate quantities of pyridoxine (vitamin B_6). The results obtained have shown that porous polyurethane/hydroxyapatite scaffolds with an interconnected network were produced and pyridoxine is incorporated in apatite layer.

Experimental part

The porous polyurethane scaffolds were prepared as presented elsewhere [11-14], by the phase inversion method using polyurethane polymer, N,N-dimethyl-formamide (DMF) as solvent, and deionized water as nonsolvent.

The supersaturated calcification solution (SCS) was prepared by dissolving the chemicals (supplied by Sigma-Aldrich, Germany) such as: $CaCl_2 \cdot 2H_2O$, $NaH_2PO_4 \cdot H_2O$ and $NaHCO_3$ in deionized water. The ion concentrations of SCS solution are 4.0 Mmol·L^{-1} Na^+, 5.0 Mmol·L^{-1} Ca^{2+}, 10.0 Mmol·L^{-1} Cl^-, 2.5 Mmol·L^{-1} $(H_2PO_4)^-$, and 1.5 Mmol·L^{-1} $(HCO_3)^-$. The M-SCS solution was prepared by adding to the original SCS solution certain quantities of vitamin B_6 (pyridoxine) (purchased from Sigma-Aldrich, Germany). All chemicals are reagent grade and used without further purification.

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The biomimetic method applied to coating polyurethane surface with hydroxyapatite layer consists in immersion of polymeric samples in SCS (or in M-SCS) solutions at 37 °C, for certain period of time. Then, the samples were rinsed with deionized water, followed by drying in air at 40 °C for 24 h.

Scanning electron microscopy (SEM) (QUANTA 200 3D Dual Beam scanning electron microscope) was used to observe the morphology and chemical composition of samples. The FT-IR spectra of all samples were recorded on a DIGILAB SCIMITAR-SERIES spectrophotometer.

Results and discussions

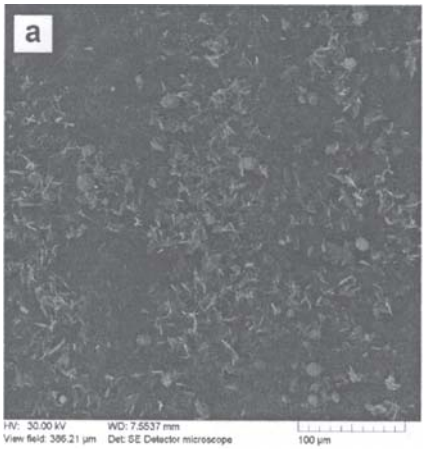
In this study we used the polyurethane porous matrices obtained by phase inversion method. These matrices have an asymmetric structure and high porosity which allows their use as scaffolds in tissue engineering [11-14].

To improve the biocompatibility and osteoinductive properties of these matrices we have achieved in this study hydroxyapatite deposition by biomimetic method. Biomimetic methods use a series of solutions that contain large amounts of calcium and phosphorus, in which is immerse the material that is intended to achieve deposition of hydroxyapatite, at temperature and pH conditions that mimic physiological conditions. Also, to these solutions can add organic matter that can facilitate faster apatite deposition.

In the present study we used vitamin B_6 to see if it has a role in the apatite deposition on the surface of polyurethane and to incorporate it into apatite layer. Vitamin B_6, also called pyridoxine, is an alcohol and it is one of 8 B vitamins. These B vitamins, often referred to as B complex vitamins, help the body metabolize fats and protein. All B vitamins are water-soluble, meaning that the body does not store them. Several studies were concluded that vitamin B_6 deficient cross-linking may be responsible for the observed delay in bone development and aforementioned cartilage histological alterations [15].

In our researches, the porous polyurethane samples are soaked in SCS or in M-SCS solutions. After 120 h of incubation, sample immersed in SCS had exhibited plate shaped hydroxyapatite crystals deposited on polyurethane surface (fig. 1).

After the same incubation time, sample immersed in M-SCS had exhibited a hydroxyapatite layer with another configuration (fig. 2). As shown in figure 2, after 120 h of immersion in M-SCS solution, on the whole polyurethane surface some flower-like apatite aggregates, with a diameter varied from 10 up to 50 μm, were visible. It can be observed that the apatite aggregates are formed from crystals in the form of thin plates. This result is due, probably, to the vitamin added.



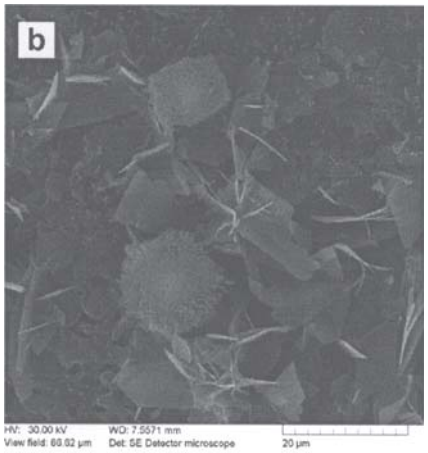
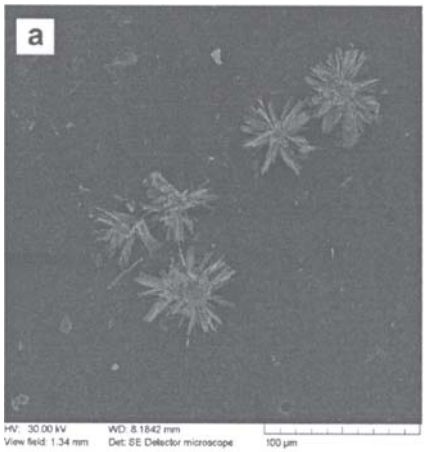


Fig. 1. SEM photographs of the surface of porous polyurethane sample (at different magnitudes) after soaking in SCS solution for 120 h at 37 °C



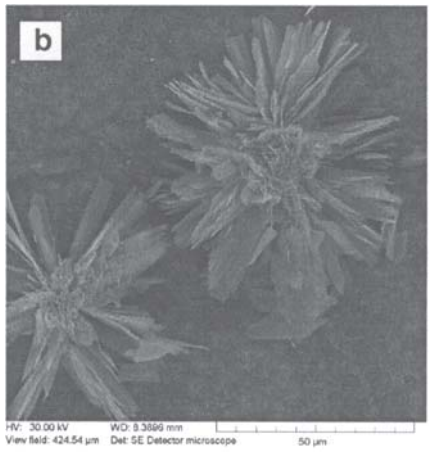


Fig. 2. SEM photographs of the surface of porous polyurethane sample (at different magnitudes) after soaking in M-SCS solution for 120 h at 37 °C

Introduction of vitamin B_6 into SCS solution leads to an increase in the particle size, but does not disturb the degree of binding of hydroxyapatite with the polyurethane support.

The formation of hydroxyapatite particles on polyurethane support was observed by FT-IR absorption studies. Figure 3 shows the FT-IR spectra of the polyurethane scaffold before and after incubation in SCS and M-SCS solutions. The specific peaks detected at 3316, 1526, 2917 and 1736 cm^{-1} were assigned to the N–H, C–N, C–H and C=O groups present in the polyurethane support. The bands at 1030 – 1150 and 920 cm^{-1} correspond to phosphate stretching vibration attributed to hydroxyapatite layer. The FT-IR spectrum of sample immersed in M-SCS show an additional band at 1650 – 1450 cm^{-1} attributed to

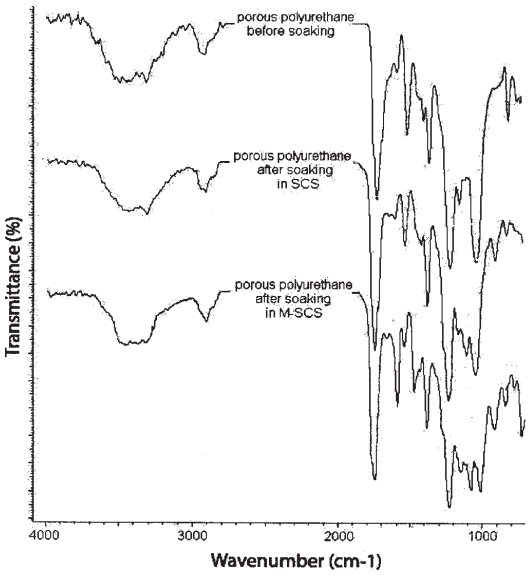


Fig. 3. FT-IR spectra of polyurethane scaffolds after soaking in SCS solution and in M-SCS solution for 120 h at 37 °C

vitamin B_6 (fig. 4) adsorbed on the polymeric and apatite surface. The peak at 1549 cm^{-1} may be assigned to the in-plane pyridine ring stretching vibration assigned to vitamin B_6 as presented in literature [16].

Therefore, considering the results presented it can be said that the biomimetic method used can make a thin layer of hydroxyapatite on polyurethane surface and the vitamin is adsorbed in this layer.

Conclusions

In this study we used the polyurethane porous matrices obtained by phase inversion method. To improve the biocompatibility and osteoinductive properties of these matrices we have achieved hydroxyapatite deposition by biomimetic method, in presence of vitamin B_6. Introduction of vitamin B_6 into a supersaturated calcification solution leads to an increase in the particle size of hydroxyapatite crystals, but does not affect the degree of binding of hydroxyapatite with the polyurethane support. The FT-IR studies indicate that in the M-SCS solution containing vitamin B_6 hydroxyapatite is deposited on polymer support and the vitamin is incorporated in this layer.

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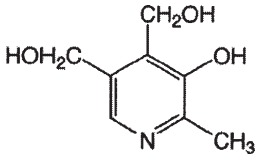

Fig. 4. Chemical structures of pyridoxine molecule

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