In this paper three new materials with 3D structure based on hydroxyapatite (HAP), \( \beta \) tricalcium phosphate (\( \beta \)-TCP) and hydroxyapatite + \( \beta \) tricalcium phosphate (HAP + \( \beta \)-TCP) were studied. The short term behaviour of these new materials in simulated body fluid (SBF) was analysed from their weight variations and structure changes. Also, the variation in time of pH and concentration of calcium and phosphorus ions from SBF was monitored for the investigation of the coupled mechanism of scaffold dissolution and calcium and phosphorus deposition. The pH of SBF increased at the beginning due to the dissolution of the soluble phases from scaffold material and then maintained at a constant value as results of a deposition process that exceeded the scaffold dissolution. Thus, the bioactivity increased in time. Concentration of the calcium and phosphorus ions from SBF decreased in the first 170 immersion hours due to the deposition process on the scaffolds; after 200 h, their concentration remained almost constantly as a result of compensation of processes of dissolution and deposition. The morpho-structural studies performed by SEM microscopy, confirmed that, after 360 immersion hours appeared a layer made of nanosized acicular grains that coats the walls of the cavities.

**Keywords:** hydroxyapatite, \( \beta \) tricalcium phosphate; hydroxyapatite + \( \beta \) tricalcium phosphate, ICP-OES; SEM

The most important problem of the tissue engineering is the obtaining of scaffolds able to guide the process of tissue regeneration. An ideal scaffold should have chemical, biological and bio-physical properties that can initiate and control the processes at cellular and tissue level [1-4].

Various materials were proposed to be used as scaffolds for tissue regeneration:

- scaffolds based on natural materials: coral [5,6]; natural matrices formed from cells of different organs [7-9], fibrin, heparin, collagen [10], peptides [11];
- scaffolds based on synthetic materials: poly(DL-lactic acid) (PLA) [12], poly(L,D-lactic-glycolic acid) (PLGA) [13], poly(L-lactic acid) (PLLA) [14,15], poly-lactide-co-glycolide (PLG) [16], poly(desaminotyrosyl-tyrosine ethyl ester carbonate (pDESC) [17], bioglass [18], carbon nano tubes [19,20], zein scaffold [21];
- semi-synthetic scaffolds: silk fibroin (SF) [22], fibroin hydrogel (FH) [23];
- hybrid scaffolds: poly(D,L-lactic-co-glycolic acid)/hydroxyapatite (PLGA/HAP) [24], polycaprolactone/hydroxyapatite (PCL/HAP) [25], medical polycaprolactone/calcium phosphate (mPCL/CaP) [26], nano-hydroxyapatite/polyamide (nHA/PA) [27], poly(D,L-lactide)/calcium carbonate (vaterite) (PDLLA/vaterite) [28].

Materials obtained from natural resources have the advantage that are biologically recognised by the respective organ, but they can not be purified from the immunology point of view [29,30]. Therefore, the synthetic or hybrid scaffolds based on hydroxyapatite [31,32], crystalline or amorphous calcium phosphate [33-36], tricalcium phosphate [37,38] are preferred because their properties can be controlled and optimised [39]: porosity, roughness, Young’s modulus, dissolution rate in tissues, etc.

In this paper three new materials with 3D structure based on hydroxyapatite (HAP), \( \beta \) tricalcium phosphate (\( \beta \)-TCP) and hydroxyapatite + \( \beta \) tricalcium phosphate (HAP + \( \beta \)-TCP) were studied. These materials are proposed to be used as orthopaedic resorbed scaffolds and it is expected to present a dissolution process, a progressive resorption simultaneously with a process of deposition of calcium and phosphorus from the human fluid, so to form new bones. The short term behaviour of these new materials with 3D structure in simulated body fluid (SBF) was analysed from their weight variations and structure changes. Also, the variation in time of pH and quantity of calcium and phosphorus ions from SBF were monitored to investigate the coupled mechanism of scaffold dissolution and calcium and phosphorus deposition.

**Experimental part**

The materials with 3D structure (scaffolds) used in experiments were: hydroxyapatite (HAP), \( \beta \) tricalcium phosphate (\( \beta \)-TCP) and hydroxyapatite + \( \beta \) tricalcium phosphate (HAP + \( \beta \)-TCP) mixture. The composition of the scaffolds is proprietary. Samples of parallelepiped form were used.

Simulated body fluid (SBF) has the composition (g/L): NaCl – 7.99; KCI – 0.224; NaHCO3 – 0.35; KHPO4·3H2O – 0.228; MgCl2·6H2O – 0.305; CaCl2 – 0.278; Na2SO4 – 0.071. Temperature was kept at 370 ±1°C.

The following experimental techniques were used: monitoring of scaffold weight, of SBF pH, induced coupled plasma–optical emission spectroscopy (ICP-OES) and scanning electron microscopy (SEM).

For the monitoring of weight, the samples were exposed for short term (360 h) in simulated body fluid at 37°C and were periodically verified after 50, 100, 170, 250, 360 immersion hours. Evaluation of the weight variation was made by the determination of the percentage variation of the weight after immersion in SBF:

\[
\Delta G = \frac{G_2 - G_1}{G_1} \cdot 100,
\]

where: \( G_1 \) is the initial weight of the sample and \( G_2 \) is the sample weight after immersion in biological fluid and
drying in air for about 2 h (till a constant weight is reached). Negative values for ΔG show the weight loss, i.e., the material is dissolved in SBF. Positive values of ΔG show a deposition from SBF.

Monitoring of pH values of the simulated body fluid for short term (50, 100, 170, 250, 360 immersion hours) was carried out with a pH-meter InoLab WTE (Germany) with a precision of ±0.01.

Induced coupled plasma–optical emission spectroscopy (ICP-OES, Perkin Elmer) was used for determination of the short term change (360 h) of the calcium and phosphorus quantity on the scaffolds.

The short term (360 h) morpho-structural changes of the scaffolds were studied by scanning electron microscopy (SEM) using the microscope EVO LS10.

Results and discussions

Short term monitoring of the scaffold weight

The variation of the weight of the materials with 3D structure determined by periodic verifications for short term, till 360 immersion hours is presented in table 1.

<table>
<thead>
<tr>
<th>Material</th>
<th>Time (h)</th>
<th>ΔG(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAP</td>
<td>50</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>170</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>5.16</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>5.16</td>
</tr>
<tr>
<td>β-TCP</td>
<td>50</td>
<td>-0.45</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-2.38</td>
</tr>
<tr>
<td></td>
<td>170</td>
<td>-2.77</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>0.32</td>
</tr>
<tr>
<td>HAP+β-TCP</td>
<td>50</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-0.13</td>
</tr>
<tr>
<td></td>
<td>170</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>2.17</td>
</tr>
</tbody>
</table>

From table 1 can be observed that:

- hydroxyapatite (HAP) presents the increase of the weight denoting that this material does not dissolve in the testing period (360 h), i.e. the calcium and phosphorus ions from SBF produce deposits on this material;
- β-tricalcium phosphate (β-TCP) weight decreased at the beginning, namely this material partially dissolves in SBF, and then its weight increased due to the deposition of calcium and phosphorus from SBF. Because the weight increases moderately, it results that a dissolution process was superimposed to the deposition process; thus, after 250 immersion hours, the deposition process is more important than the dissolution process;
- hydroxyapatite + β-tricalcium phosphate (HAP + β-TCP) exhibited a dissolution process at the beginning because its weight variation presents negative values; then, a deposition process, that takes concomitantly place with the dissolution process, is more intense conducting to positive values of ΔG parameter.

Short term monitoring of SBF pH values

The evolution of SBF pH values in which the scaffold of HAP type was immersed (fig. 1) presented in the first 170 h a slight increase from the initial value of 7.75 up to 8.53 due to the low dissolution of its soluble phases [32]; after that, the pH values tend to be constant suggesting that the material porosity is very proper for bone deposition (bioactivity) [32,33].

In the case of scaffold of type β-TCP (fig. 1), the pH increase is a little more marked from the initial value of 7.75 up to 8.64, signifying an important dissolution of the constituent phases. The fact that, after 170 immersion hours in SBF, the pH values become almost constantly, around the value of 8.55, denote that the process of calcium and phosphorus deposition was performed, so, the scaffold has a bioactive behaviour [32].

The same pH evolutions were registered for scaffold of type HAP + β-TCP (fig 1): in the first 170 immersion hours the pH value increased from 7.75 to 8.55, due to the same dissolution process of the constituent phases; however, the slight decrease followed by the stabilization of the pH to 8.5 value shows that, after 200 h, the deposition process exceeds the dissolution process, and therefore, the material bioactivity increases.

Comparing the results obtained for the three investigated materials (fig. 1) it can be observed a similar variation of the pH values; the lowest pH increase appeared in the case of HAP type scaffold and the highest pH increase in the case of β-TCP type scaffold, proving that the β-TCP type scaffold is more soluble.

Short term monitoring of calcium and phosphorus concentration from SBF

The concentration of calcium and phosphorus ions from SBF decreased very much in the first 170 immersion hours of HAP (figs. 2 and 3) due to the deposition on the scaffold. It was showed that these ions penetrate into the material pores and progressively nucleate and crystallised as apatite [32]. After 200 h, the concentration of these ions remains almost constantly, meaning that the simultaneous processes of dissolution and deposition are compensated.

As figures 2 and 3 show, a lower decrease of the calcium and phosphorus ion concentration in SBF was registered for both β-TCP type scaffold and for HAP + β-TCP type scaffold due to the fact that in the case of these materials,
the dissolution and deposition processes take concomitantly place, namely, the material resorption is coupled with the deposition of calcium and phosphorus and therefore with the bone formation [38,39].

**Short term monitoring of the scaffold morfo-structure**

The scaffold surface morphology and its evolution in time were studied by scanning electron microscopy (SEM). The initial and periodical verifications pointed out the processes that took place on the sample surfaces.

The initial SEM images of scaffolds (fig. 4) illustrate the sponge-like architecture of non-oriented interconnected cavities in a three-dimensional open network.

Immersion in simulated body fluid (SBF) for 360 immersion hours (fig. 5) clearly affects the surface morphology. Deposition from SBF is clearly demonstrated on all three materials. SEM images reveal a layer that coats the walls of the cavities, made of nanosized acicular grains.

**Conclusions**

The short term variations of the scaffold weight immersed in simulated body fluid revealed the fact that simultaneously with the dissolution process of the studied materials calcium and phosphorus were deposited, especially in the first 170 immersion hours. As a consequence, these materials are bioactive and promote the bone formation.

The pH of SBF increased at the beginning of scaffold immersion due to the dissolution of the soluble phases from these materials and then maintained at a constant value as result of the calcium and phosphorous deposition process that exceeded the scaffold dissolution process; so, the bioactivity increased in time.

Concentration of the calcium and phosphorus ions from SBF decreased in the first 170 immersion hours due to their consumption in the layer deposition on the scaffolds; after 200 hours, both concentrations remained almost constantly as a result of compensation during dissolution and deposition; on the whole, the bone formation takes place.

The morpho-structural studies by SEM microscopy confirmed that, after 360 immersion hours appeared a layer that coats the walls of the cavities, made of nanosized acicular grains.

**Acknowledgements:** Research founded by Romanian National Research, Developed and Innovation Program – PN II project number 41-059/2007. The authors gratefully acknowledge to chemist I. Dumitriu from ICECHIM, Bucharest, Romania, for ICP-OES measurements.
References

Manuscript received: 27.04.2010

REV. CHIM. (Bucharest) ● 62 ● No. 2 ● 2011 http://www.revistadechimie.ro