

## **Preparation and characterization of new hybrid nanostructured thin films for biosensors design**

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### **Abstract**

The present paper reports on an innovative route for the preparation of new hybrid nanostructured thin films based on hydroxyapatite and functionalized polyurethane. Hybrid nanopowders based on hydroxyapatite and functionalized polyurethane have been synthesized by a hydrothermal method with high pressure and low temperature conditions and further used for spin coating deposition. Biocompatible thin films with a thickness of about 50 nm have been deposited onto Si/SiO<sub>2</sub>/Ti/Au substrates and their properties recommend them suitable as possible electrodes for the fabrication of impedance biosensors. Hybrid materials with improved properties are obtained, combining the mechanical properties of polyurethane with biocompatible properties of hydroxyapatite (bioactivity and osteoconductivity). The presence of functional groups in polyurethane structure ensures the existence of strong interactions between components and an increased affinity of the thin films for further protein bonding in biosensor design. Hybrid nanostructured thin films based on hydrothermally synthesized hydroxyapatite–polyurethane nanopowders could enhance the amount of immobilized biomolecules in the construction of an impedance biosensor for diagnosis and therapy of bone diseases.

## **1. Introduction**

Hydroxyapatite has been extensively studied as coating material for biosensor applications due to its excellent biocompatibility and bioactivity, forming strong bonds to bone tissue [1–4]. Coatings can be produced by ion sputtering, plasma spraying, sol–gel, electrolysis, biomimetic methods, spin coating, electrophoretic deposition, etc. [2,3]. Recently, biosensors based on hydroxyapatite–polymer composite have been developed [5,6]. To our knowledge, HAp–PU composites were previously prepared by adding inorganic component as ceramic filler, during polyurethane synthesis or mechanical mixing. Also, HAp–PU coatings were obtained by biomimetic deposition of hydroxyapatite on polyurethane surface, in SBF at 37 °C. The present paper studies a new route for the preparation of hybrid nanostructured thin films based on hydroxyapatite–polyurethane (HAp–PU). For the first time, specially designed polyurethane with COOH groups on its surface was used for high pressure hydrothermal synthesis of nanostructured hybrids. It was previously reported [7–10]. that high pressure plays an important role in the formation of strong interactions between inorganic and organic components. As a novelty, possible interactions between the two components of the hybrid material were established using Molecular Dynamics (MD) simulations at high pressures. The goal of the paper is to demonstrate the potential of hydrothermal synthesis followed by spin coating deposition to obtain biocompatible thin films for the design of biosensors. Consequently, *in vitro* measurements using mouse neuroblastoma cells were performed and potential use of HAp–PU coatings as materials for spinal cord injury will be addressed in the future.

## **2. Materials and methods**

Table 1 presents the experimental conditions for the synthesis of nanostructured hybrid powders which were further used for spin coating process. HAp–PU nanopowders obtained by hydrothermal method were dispersed in water and stabilized as colloidal suspensions by the aid of a dispersing agent. Stable aqueous suspensions of hybrid nanostructured powders were spin coated onto Si/SiO<sub>2</sub>/Ti/Au substrates with 12 mm diameter and 500 μm thickness.

Spin coating deposition was performed on a KW-4A spin coater at 550 rpm for 9 s followed by 1880 rpm for 10 s.

**Table 1**  
Hybrid nanostructured thin films.

Thin film name	Substrate	Type of coated material	Hydrothermal synthesis conditions of the hybrid nanostructured powder used as coating material	
			P, atm	T, °C
TF1	Si/SiO <sub>2</sub> /Ti/Au	HAp-PU hybrid	40	100
TF2	Si/SiO <sub>2</sub> /Ti/Au	HAp-PU hybrid	60	100
TF3	Si/SiO <sub>2</sub> /Ti/Au	HAp-PU hybrid	80	100

## 2.1. Characterization methods

The proliferation of mouse neuroblastoma (N2a) cells on n-HA/PU scaffold was determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazoliumbromide) assay. At 24 h after seeding, the culture medium was replaced with 100 µl of MTT solution and the hybrid thin film samples were incubated for 1.5 h at 37 °C in 5% CO<sub>2</sub>. Afterwards, the MTT solution was replaced with 100 µl of dimethylsulphoxide and the multiwell plate was mixed until complete salt crystal dissolution was obtained. Absorbance was measured using a BioTek PowerWave 340 microplate reader at a wavelength of 560 nm. The data were analyzed statistically using analysis of variance (ANOVA)

## 2.2. Molecular Dynamics simulations

Molecular Dynamics simulations were performed using AMBER 10 software [11]. The initial structure of PU was generated as a bulk configuration of this polymer by Amorphous Cell module of Materials Studio software package from Accelrys Inc. The (1 1 0) HAp surface (55 Å × 65 Å × 15 Å) was also generated using Materials Studio. These atomic type of models were consequently parameterized for the AMBER calculation using an Antechamber suite. General Amber Force Field (GAFF) was used to assign all the relevant bond, nonbond parameters [12]. Hap surface was fixed during simulations. SHAKE algorithm was used to constrain hydrogen stretching and to allow for an 2 fs integration step [13]. The weak-coupling algorithm was used to ensure constant temperature [14]. Pressure coupling algorithm as described also in [14] was used as the barostat.

## 3. Results and discussion

### 3.1. Molecular Dynamics simulations

Molecular Dynamics simulations were used in order to study some possible interactions between  $\text{Ca}^{2+}$  ions (that built in hydroxyapatite and that free ones) and  $\text{COO}^-$  groups of the polyurethane at different pressures (20 atm and 80 atm, respectively). The results of 7 ns of MD simulation between hydroxyapatite and polyurethane in explicit salt water ( $I_S = 0.15 \text{ M}$ ) are illustrated in Fig. 1. This particular simulation was carried out with pressure set to 20 atm and temperature 373 K. The HAp-PU interaction mainly via  $\text{Ca}^{2+}$  ions and  $\text{COO}^-$  groups is also clearly evident from time averaged (over last 2 ns) radial distribution function of  $\text{COO}^-$  oxygens of PU respect to  $\text{Ca}^{2+}$  ions from HA. Positionally stable  $\text{COO}^-$  oxygens were identified at a distance around  $2.5 \text{ \AA}$  from the closest surface  $\text{Ca}^{2+}$  ion. The strong interaction of  $\text{Ca}^{2+}$  with  $\text{COO}^-$  groups from PU was simultaneously verified also by simulation of standalone PU and free  $\text{Ca}^{2+}$  ions again in explicit salt water under 20 and 80 atm and  $T = 373 \text{ K}$ . This, 10 ns long simulation is illustrated in Fig. 2. A shrinkage of the polymer chain is observed, leading to a more compact structure, partially because of the high pressure but also because of the condensation of the  $\text{Ca}^{2+}$  ions. Similarly as in the case of the HAp-PU interaction study, simulations revealed very stable locations of the  $\text{Ca}^{2+}$  ions at the distance of cca  $2.4 \text{ \AA}$  from the nearest oxygen of the  $\text{COO}^-$  group. This stability is a little better for the pressure of 80 atm otherwise there are no remarkable differences between 20 and 80 atm from the point of view of MD simulation.

### **3.2. Biocompatibility tests: MTT assay**

The MTT assay was performed to assess the viability and the proliferation of the cells on the HAp-PU thin films surface. The experimental data obtained on multiple sets of TF1, TF2 and TF3 thin films, respectively were statistically analyzed using ANOVA method and Graph pad prism software. The results are presented in Fig. 3. All the samples are comparable with the control. The tendency of cell viability is better in the case of sample TF2. Sample TF3 presents also good cell viability. Relatively good cell viability is observed in the case of a TF1 sample. The results are in accordance with MD simulations. Higher pressure leads to stronger interactions between organic (PU) and inorganic components (HAp) but there are no significant differences between 60 and 80 atm. Better properties of the hybrid coatings are expected due to their unique structure, compared to HAp-PU coatings obtained in the literature using biomimetic or mechanical methods. Cells viability of the hybrid nanostructured thin films is comparable with the control, suggesting that TF surfaces could be used as bioactive substrate for the design of new biosensors.

#### **4. Conclusions**

Hybrid nanostructured thin films based on hydroxyapatite and specially designed polyurethane were prepared using hydrothermal synthesis at high pressure followed by spin coating deposition. For the first time, hydroxyapatite–polyurethane hybrids were obtained in high pressure conditions, dispersing the organic component (hard type polyurethane) into aqueous solution of hydroxyapatite precursor. A homogenous hybrid structure is obtained and each inorganic “nano-whisker” is surrounded by polyurethane matrix. In vitro tests of HAp–PU thin films presented were performed on mouse neuroblastoma cells (N2a) aiming to investigate their potential for spinal cord injury diagnosis/therapy. Biocompatible HAp–PU layers with strong interactions between Ca<sup>2+</sup> ions and COO<sup>-</sup> groups present a great potential for further protein binding in new biosensor design.

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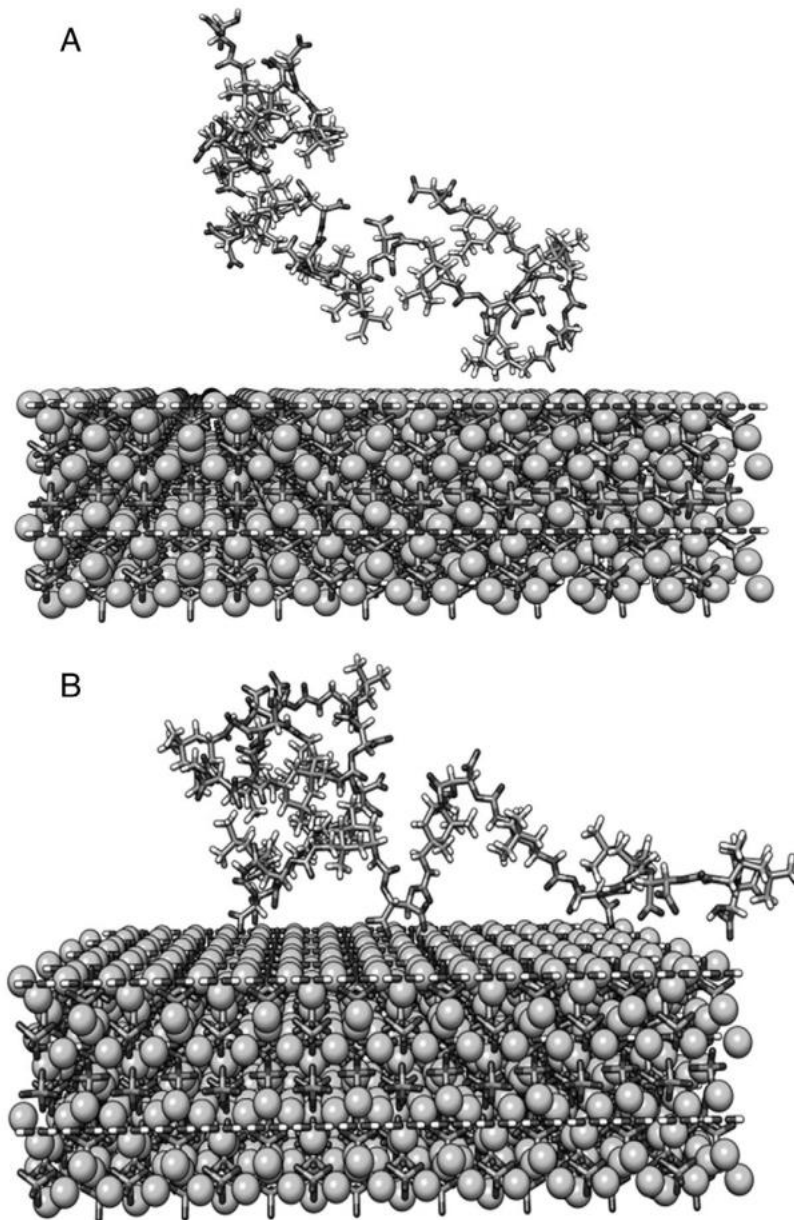


Fig. 1. Simulation of HAp–PU interactions at  $P=20$  atm,  $T=373$  K, and  $IS=0.15$  ( $IS$ =ionic strength). a) Start of the simulation and b) end of the simulation.

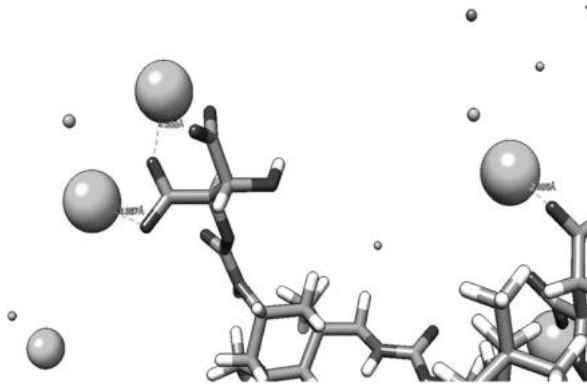


Fig. 2. Simulation of  $\text{Ca}^{2+}$ -PU interactions at 20 and 80 atm. The  $\text{Ca}^{2+}$  ions are trapped at the distance around 2.4 Å from the nearest  $\text{COO}^-$  oxygens.

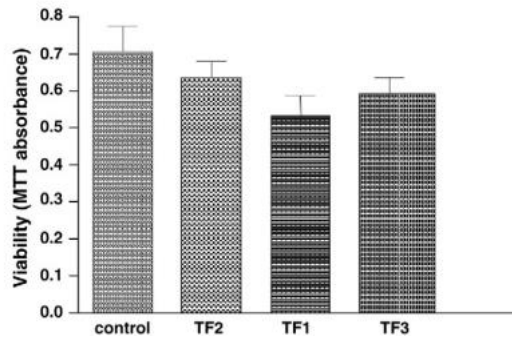


Fig. 3. MTT assay. ANOVA one way analysis of variance.