SYNTHESIS AND CHARACTERIZATION OF NOVEL α–CHITIN/ZIRCONIA COMPOSITES

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Abstract
A new method for the synthesis of chitin-ZrO_2 biocomposite is proposed. The organic matrix was a chitin skeleton obtained by chemical extraction from marine sponges. Synthesis of the chitin-ZrO_2 biocomposites was realised in two steps. At first ZrO_2 nanoparticles were synthesised by the sol-gel method. In the second step chitin matrix obtained by chemical extraction from marine sponge, was introduced into a glass ampoule to which zirconia dispersion was added. The hybrids obtained were subjected to thermal analysis (TG), chemical composition evaluation (EDS) and SEM analysis.

1 Introduction

Dynamic technological progress and rapid development of nanotechnology, biomedicine and biotechnology have stimulated the demand for new functional materials of specific, strictly defined properties. Metals, ceramic or polymers in their pure form do not meet the rigorous requirements for certain target application. For this reason in many research centres much interest has been devoted to syntheses and characterisation of new hybrid materials of unique properties achieved thanks to a combination of organic and inorganic components. The use of carefully selected components permits getting materials fit for particular target application [1]. For example biomaterials designed for biomedicine should be nontoxic, biodegradable, high surface area and porosity adjusted for particular application [2-5]. Such materials are used in many fields of biomedicine, including tissue engineering [2,3], production of wound dressing [4] and enzyme immobilisation [5]. Fortunately, we are able to resort to many biodegradable high-molecular compounds of natural origin such as hyaluronic acid, collagen, chitin and others, which surely are biocompatible and undergo controlled degradation.

Chitin is a biopolymer found in skeletons of arthropods, molluscs, sponges and fungi, built of *N*-acetyl-D-glucozoamine mers linked by β-1,4-glycoside bonds. The properties of chitin make it attractive for many branches of industry. Recently, high adsorption capacity of chitin towards organic dyes [7,8] and heavy metals [9,10] has been proved, which would permit its effective application for waste and industrial wastewater purification. Owing to high affinity to proteins, chitin has found application in drug distribution systems and production of biosensors. From the biomedical viewpoint the attractive properties of chitin are

biodegradability, biocompatibility and nontoxicity, but its drawbacks are poor solubility and poor mechanical strength, so hitherto not many papers on the syntheses of 3D inorganic/organic chitin materials have been published. Ehrlich and co-workers have reported the presence of three-dimensional chitin scaffolds as an integral part of skeleton in several demosponges [11-13] and some glass sponges [14]. Ehrlich has proved that the presence of inorganic minerals in combination with chitin matrix ensures proper stability, flexibility and mechanical strength of skeletons of the organisms studied [15]. Jayakumar in a series of papers has described the method of preparation of chitin composites for potential application in bone regeneration [16-18]. This author has obtained chitin scaffolds by addition of inorganic oxides, mainly SiO_2 [16], TiO_2 [17] and ZrO_2[18] to chitin hydrogel. The use of lyophilisation permitted getting a 3D skeleton of high porosity which ensures good diffusion of oxygen and nutrients and leads to successful proliferation of bone-restoring cells. The so far published results have proved that the use of inorganic fillers in combination with chitin gives nontoxic, biocompatible materials characterised by a controllable biodegradation time. Introduction of inorganic oxides permits achievement of high bioactivity and improved mechanical properties.

Zirconium dioxide, known also as zirconia, is a ceramic materials of a wide gamut of applications related to its attractive properties. Its exceptionally low thermal expansion coefficient, high thermal and chemical stability favour its application in production of ceramic and insulating materials [19]. Thanks to its amphoteric character and red-ox properties it is used as a catalyst or a catalytic support in organic synthesis [20].] Thanks to its ability to form complexes with amine and carboxylic groups present in the enzyme molecules zirconia is used for enzyme immobilisation and production of biosensors [21-24]. Recent studies realised by independent research teams indicate ZrO_2 as a important component of renewable, electrodes used in electrochemical biosensing of DNA hybridisation [22-24]. Moreover, the photoluminescent properties of monodisperse spherical particles of zirconia permit its application in cheap, efficient and environmentally-friendly luminescent materials [25].

Summarising, combination chitin with inorganic zirconium oxide could lead to development of novel three-dimensional materials with unique mechanical and biological properties.

The aim of this study was to develop a new method for the synthesis of three-dimensional chitin-zirconia composites by incorporation of zirconia into chitinous matrix isolated from *Ianthella basta* and *Aplysina cauliformis* skeletons.

2 Materials and testing methods

2.1 Isolation of three-dimensional chitin matrix

Three-dimensional α-chitin network was isolated from *Ianthella basta* and *Aplysina cauliformis* by the method described by Ehrlich and co-workers [11-13]. In brief, samples of dried sponge skeleton were immersed in 2.5 M NaOH at 37°C. After 72 h, the product was isolated and washed in distilled water. At the next step the brownish chitinous scaffold was immersed in 20% acetic acid. Afterwards the sample was rinsed three times with water and again immersed in 2.5 M NaOH solution for 24 h. This procedure was repeated until the obtained chitinous material was absolutely transparent. Sodium hydroxide and acetic acid of reagent grade were purchased from Sigma-Aldrich Co.

2.2 Preparation of chitin-zirconia materials by two-step method

The synthesis of chitin-ZrO_2 biocomposite was performed in two steps. At first zirconia dispersion was synthesised by the procedure reported by Widoniak et al. [26]. In brief, 100 ml of anhydrous ethanol and 0.4 ml NaCl aqueous solution were placed in a three-necked flask under vigorous stirring. Then, 3.25 ml $Zr(OPr)_4$ was added to the above solution at room

temperature under N_2 atmosphere. At the next step, the chitin matrix obtained as a result of chemical extraction of sea sponges *Aplysina cauliformis* (UZr60i) and *Ianthella basta* (UZr20i) was introduced into an ampoule and flooded with the earlier obtained dispersion. The ampoule was closed and incubated at 60°C. After 24 h the chitin fragment was isolated, washed with anhydrous ethanol and dried by the convection method at 105°C.

2.3 Analytical techniques

Microstructure and morphology of the samples obtained were analyzed using a Scanning Electron Microscope (SEM) (Zeiss EVO40). Thermal analyses of chitin-zirconia biocomposites were carried out with Jupiter STA 449F3–Netzsch analyser with Al_2O_3 crucible. Measurements were performed in nitrogen atmosphere at the heating rate of 10°C min^{-1}. The samples were heated to 900°C, starting from 30°C. Elemental composition of obtained materials was evaluated using an energy dispersive X-ray analysis (EDS) (VEGA - TESCAN).

3 Results and discussion

The results obtained in this study results have shown that both demineralised chitinous sponge skeletons could be effectively remineralised by zirconia particles produced in a modified sol-gel process. Analysis of SEM images indicates that the method proposed gives three-dimensional biomaterials with homogenous distribution of zirconia over both chitin templates (Figure 1 a,b).

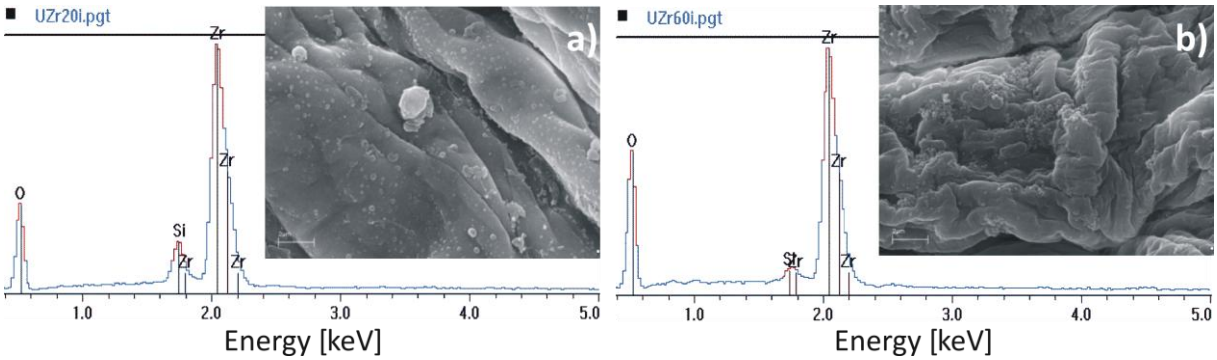

Figure 1. EDS spectra and SEM micrographs of chitin-zirconia composites obtained by two-step incorporation of zirconia into three-dimensional chitin matrix of: (a) *Ianthella basta* and (b) *Aplysina cauliformis* demineralized skeletons

As follows from SEM images, the zirconia particles deposited on chitin matrix are characterised by regular, spherical shape and have diameters from the range 100-300 nm which was confirmed by measurements realised with a Zetasizer Nano ZS analyser (Figure 2). Homogeneous distribution of zirconia is confirmed by a relatively low coefficient of polydispersity index (PdI=0,375). Zirconia incorporation into three-dimensional chitinous matrices was also confirmed by EDS analyses; the representative spectra (Figure 1) show the presence of zirconium and oxygen peaks. The trace amount of silicon evidenced by the spectra can testify to incomplete demineralisation of sea sponges skeletons, however, the presence of silicon has no significant effect on zirconia incorporation into a 3D chitinous matrix.

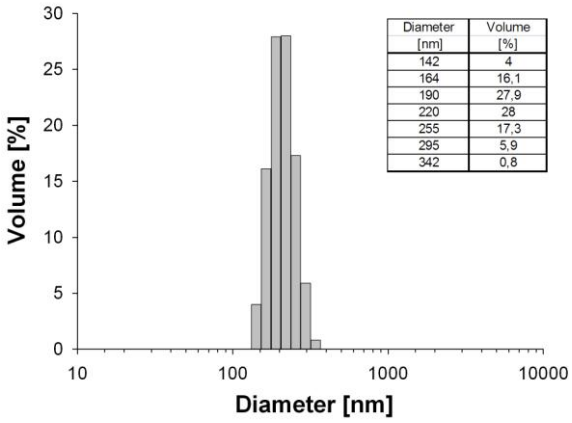

Figure 2. Particle size distribution of zirconium oxide deposited over the chitin surface

In order to establish the ranges of temperatures corresponding to important chemical transformations in chitin matrices and chitin-zirconia composites, the proper samples were subjected to thermogravimetric measurements. The TG curves illustrating thermal degradations of non-mineralized chitin scaffolds and chitin-zirconia composites are presented below (Figure 3). No significant differences were noted between the TG curves recorded for chitin from *Aplysina cauliformis* and *Ianthella basta*. According to the results, the main degradation process of both of non-mineralized chitin skeletons starts at about 275°C. It is in close relation with Ehrlich results which indicate chitinous skeletons of poriferan origin as a good template for hydrothermal reaction conditions [12-15]. The TG curves of the samples of chitin-zirconia composites show a rapid weight loss at about 270°C corresponding to removal of chitin template. The TG profiles of chitin-zirconia composites presented in Figure 3 imply that the inorganic content was of about 50 and 55% for UZr20i and UZr60i samples, respectively. These results confirm the effectiveness of the proposed method of chitin-zirconia composite production.

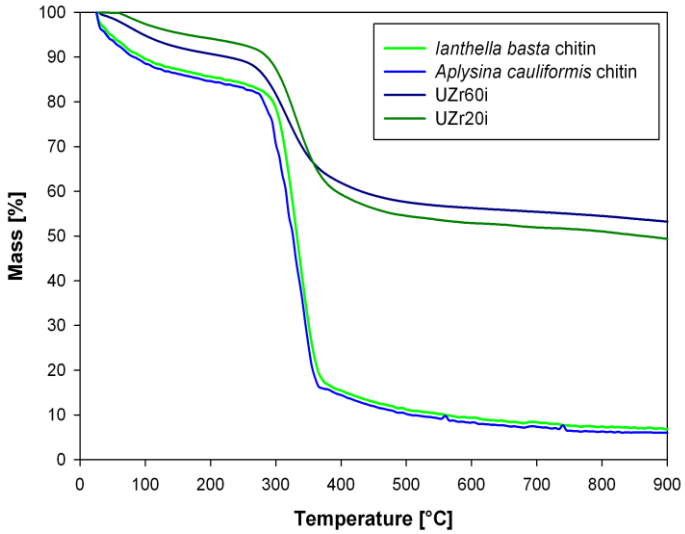

Figure 3. TG curves of nonmineralized and mineralized chitinous scaffolds of *Ianthella basta* and *Aplysina cauliformis*.

Conclusions

The present work describes the proposed method for the synthesis of novel hybrid chitinous organic-inorganic materials by incorporation of zirconia into three-dimensional chitinous skeletons of *Ianthella basta* and *Aplysinia cauliformis.* On the basis of SEM micrographs and elemental analyses it can be concluded that α-chitin as a good organic template for zirconia deposition. The compatibility of hydroxyl groups of chitin with zirconia particles permitted getting chitin-zirconia composites with homogenous deposition of regular spherical-shaped zirconia particles over chitin template. The chitin-ZrO_2 biocomposites obtained are promising for future biomedical applications (enzyme immobilisation) and chemical catalysis, which requires further studies.

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References

[1] Kickelbick G. *Hybrid Materials: Synthesis, Characterization and Applications* Wiley-VCH, Weinheim (2007).

[2] Wang M. Developing bioactive composite materials for tissue replacement, *Biomaterials*, **24**, pp. 2133-2151 (2003).

[3] Jayakumar R., Prabaharan M., Sudheesh Kumar P.T., Nair S.V., Tamura H. Biomaterials based on chitin and chitosan in wound dressing applications, *Biotechnology Advances* **29,** 322-327 (2011).

[4] Tamura H., Furuike T., Nair S.V., Jayakumar R. Biomedical applications of chitin hydrogels and scaffolds, *Carbohydrate Polymers* **84**, 820-824 (2011).

[5] Yang T.L. Chitin-based materials in tissue engineering: applications in soft tissue and epithelial organ, *International Journal of Molecular Sciences* **12**, 1936-1963 (2011).

[6] Krajewska B. Application of chitin- nad chitosan-based materials for enzyme immobilizations: a review, *Enzyme and Microbal Technology* **35**, 126-139 (2004).

[7] Akkaya G., Uzun I., Güzel F. Kinetics of the adsorption of reactive dyes by chitin, *Dyes and Pigments* **73**, 168-177 (2007).

[8] Tang H., Zhou W., Zhang L. Adsorption kinetics studies of malachite green on chitin hydrogels, *Journal of Hazardous Materials* **209-210**, 218-225 (2012).

[9] Pinto P.X., Al-Abed S.R., Reisman D.J. Biosorption of heavy materials from mining influenced water onto chitin products, *Chemical Engineering Journal* **166**, 1002-1009 (2011).

[10] Trimukhe K.D., Varma A.J. Complexation of heavy metals by crosslinked chitin and its deacetylated derivatives, *Carbohydrate Polymers* **71**, 66-73 (2008).

[11] Brunner E., Ehrlich H., Schupp P., Hedrich R., Hunoldt S., Kammer M., Machill S., Paasch S., Bazhenov V.V., Kurek D.V., Arnold T., Brockmann S., Ruhnow M., Born R. Chitin-based scaffolds are an integral part of the skeleton of the marine demosponge Ianthella basta, *Journal of Structural Biology* **168**, 539-547 (2009).

[12] Ehrlich H., Maldonaldo M., Spindler K.D., Eckert C., Hanke T., Born R., Goebel C., Simon P., Heinemann S., Worch H. First evidence of chitin as a component of the skeletal fibers in marine sponges. Part I Verongidae (Demospongia: Porifera), *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* **308B**, 347-356 (2007).

[13] Ehrlich H., Ilan M., Maldonaldo M., Muricy G., Barastrello G., Kljajic Z., Carballo J.L., Schiaparelli S., Ereskovsky A., Schupp P., Born R., Worch H., Bazhenov V.V., Kurek D., Verlamov V., Vyalikh D., Kummar K., Sivkov V.V., Molodtsov S.L., Meissner H., Richter G., Steck E., Richter W., Hundolt S., Kammer M., Paash S., Krasokhin V., Patzke G., Brunner, Three-dimmensional chitin-based scaffolds from Verongida sponges (Demospongiae: Porifera). Part I. Isolation and identification of chitin. *International Journal of Biological Macromolecules* **47**, 132-140 (2010).

[14] Ehrlich H., Krautter M., Hanke T., Simon P., Knieb C., Heinemann S., Worch H. First evidence of the presence of chitin in skeletons of marine sponges. Part II glass sponges (Hexactinellida: Porifera), *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* **308B**, 473-483 (2007).

[15] Ehrlich H., Janussen D., Simon P., Bazhenov V.V., Shapkin N.P., Erler C., Mertig M., Born R., Henemann S., Hanke T., Worch H., Vournakis J.N. Nanostructural organization of naturally occurring composites-Part II: Silica-chitin-based biocomposites *Journal of Nanomaterials* **2008** (2008).

[16] Madhumathi K., Sudheesh Kumar P.T., Kavya K.C., Furuike T., Tamura H., Nair S.V., Jayakumar R. Novel chitin/nanosilica composite scaffolds for bone tissue engineering applications *International Journal of Biological Macromolecules* **45**, 289-292 (2009).

[17] Jayakumar R., Ramachandran R., Divyarani V.V., Chennazhi K.P., Tamura H., Nair S.V. Fabrication of chitin-chitosan/nano TiO_2-composite scaffolds for tissue engineering applications *International Journal of Biological Macromolecules* **48**, 336-344 (2011).

[18] Jayakumar R., Ramachandran R., Sudheesh Kumar P.T., Divyarani V.V., Chennazhi K.P., Tamura H., Srnivasan S., Chennazhi K. P., Nair S.V. Fabrication of chitin-chitosan/nano ZrO_2 composite scaffolds for tissue engineering applications *International Journal of Biological Macromolecules* **49**, 274-280 (2011).

[19] Girolamo G. D., Marra F., Blasi C., Serra E., Valente T. Microstructure, mechanical properties and thermal shock resistance of plasma sprayed nanostructured zirconia coatings, *Ceramics International* **37**, 2711-2717 (2011).

[20] Chen A.J, Wong S.T., Hwang C.C., Mou C.Y. Highly efficient and regioselective Halogenation over well dispersed Rhenium-promoted mesoporous Zirconia, *ACS Catalysis* **1**, 786-793 (2011).

[21] Sumana G., Das M., Srivastava S., Malhotra B.D. A novel urea biosensor based on zirconia, *Thin Solid Films* **519**, 1187-1191 (2010).

[22] Zuo S.H., Zhang L.F., Youan H.H., Lan M.B., Lawrance G.A., Wei G. Electrochemical detection of DNA hybridization by using a zirconia modified renewable carbon paste electrode, *Bioelectrochemistry* **74**, 223-226 (2009).

[23] Yang J., Wang X., Shi H., An electrochemical DNA biosensor for highly sensitive detection of phosphinothricin acetyltransferase gene sequence based on polyaniline-(mesoporous nanozirconia)/poly-tyrosine film, *Sensors and Actuators B: Chemical* **162**, 178-183 (2012).

[24] Liu B., Hu J., Foord J.S. Electrochemical detection of DNA hybridization by zirconia modified diamond electrode, *Electrochemistry Communications* **19**, 46-49 (2012).

[25] Zhang C., Li C., Yang J., Cheng Z., Hou Z., Fan Y., Lin J. Tunable luminescence in monodisperse zirconia spheres *Langmuir* **25**,7078-7083 (2009).

[26] Widoniak J., Eiden-Assmann S., Maret G. Synthesis and characterization of monodisperse zirconia particles, *European Journal of Inorganic Chemistry* **15**, 3149-3155 (2005).