DEVELOPMENT OF GRAPHENE-BASED BIOSENSOR FOR MEDICAL DIAGNOSTICS

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Abstract

The explosion of information provided by the "-omics," (genomics, proteomics, etc.) has resulted in a pressing need to develop matching diagnostic technologies, so-called biosensors. Rapid, sensitive, selective, and cost-effective analysis of different biomolecules and microorganisms is crucial in clinical diagnosis and efficient treatment of patients. Further, there is a growing demand for decentralized laboratory methodologies that can be implemented in doctor's office, emergency room or in the field for the analysis of such analytes as DNA, RNA, proteins, antibodies, bacteria, viruses, small compounds etc. Lab-on-a-chip platforms and miniaturized point-of-care devices based on biosensors fulfill these demands and are foreseen to revolutionize the future of medical diagnostics. Because of excellent electric and optical properties, graphene has recently found to be highly attractive in biosensing applications and may thrust new possibilities into the field of miniaturized medical diagnostic devices. The main objective of this project is to develop a multifunctional graphene biosensor for effective electrochemical detection of specific DNA microbial targets in biological samples. Novel nanocomposites consisting of chitosan and nanoparticle-modified graphene will be combined with locked nucleic acid molecular beacons with the goal of producing "ink" for ultrasonic non-contact printing of electrical circuits. The developed technology will allow fabrication of low cost, highly sensitive biosensors for point-of-care diagnosis.

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CHEMICAL PURITY OF NEW SEGMENTED POLYESTER BIOMATERIALS

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Introduction

Chemical purity is the crucial property of polymers for biomedical applications. All materials before in vitro testing and especially clinical studies needs to be purified and characterized with respect to potential leachable substances. The characterization pathway is described in PN-EN ISO: 10993 12:2009 standard, part 12, 13 and 18 1–3 and PN-83/P-04607 4.

In this research project new multiblock copolymer are developed, as potential materials for producing elements of extracorporeal heart assisting devices. Currently used polyurethanes (PU) possess following advantages: blood compatibility, transparency and easy processing, but their main drawbacks are poor mechanical stability and number of significant chemical changes on the polymer surface 5. Due to the disadvantages of commercially available PUs we proposed new multiblock copolymer consist from poly(ethylene terephthalate) (PET) hard segments and ethylene ester of dilinoleic dimer acid as soft segments (DLA). The aim of this work was to establish purification methodology of new PET-DLA copolymer and evaluate their chemical purity ,as potential materials for blood contacting product. A detailed characterization of physical and chemical properties of aqueous and non- polar extracts, as well as the purified product was performed.

Experimental

Materials

PET-DLA copolymer with the 50:50 hard:soft segments ratio (wt%) was obtained by two step polycondensation method. Briefly, transesterification between dimethyl terephthalate and ethylene glycol was carried out at the temperature range 150-190°C, then dimer fatty acid (DLA) was added and polycondensation reaction was carried at p=0,4mbar and temperature 255-260°C. α-Tocopherol was used as natural thermal stabilizer. The intrinsic viscosity of 0,724 dl/g was measured, and the melting temperature of 198°C was determined. The proposed chemical structure is demonstrated in FIG. 1.

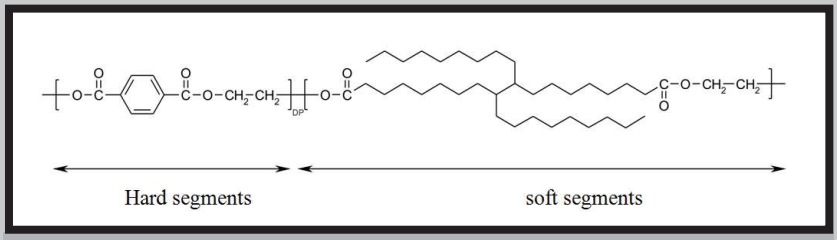

FIG. 1. Chemical structure of PET-DLA copolymer; DP - degree of polymerization for 50:50 copolymer 2,98.

Methods

Organic solvent extraction

Different alcohols: ethanol, methanol and isopropanol were examined as polar solvents. As a non-polar solvent for leachable substances, we used petroleum ether (boiling temperature 40-60°C). The extraction was carried out in Soxhlet apparatus according to the PN/P-04607:1983 and PN-EN ISO 10993-12: 2009 standards.

Water extraction

Water extraction was carried out on material previously purified by solvent extraction. Polymer granules were immersed in water at 37°C for 3, 7, 14, 21 and 28 days in shaking incubator. As reference material, commercially available thermoplastic elastomer polycarbonate polyurethane (PCU) was used. Water extracts were used for determination of total organic carbon (TOC), turbidity, conductivity and total dissolved solids.

Results and discussion

Four different solvents were used for Soxhlet extraction, and UV-Vis spectra of model solutions reflecting the main components of new polymers (thermal stabilizer, α-tocopherol, and dimethyl terephthalate, DMT) and the extracts are presented in FIG.2.

The absorbance spectra of all solutions shows maximum between 200 and 300 nm (200, 240, 280nm). This absorbance region is characteristic for π- π* absorption in aromatic compounds. The analysis of the polymer composition suggests that polymer extract contains unreacted DMT or short oligomers and/or α-tocopherol (FIG. 2b).

Total carbon (TC) analysis was carried out on purified and the neat materials and TC values were calculated according the equation: TC=TOC+IC, where: TC- total carbon amount (mg/dm^3), TOC- total organic carbon (mg/dm^3), IC- inorganic carbon (mg/dm^3), respectively. The results of total dissolved solids (TDS) and the conductivity of water extracts are presented in FIG. 3.

The obtained results allow to draw the following conclusions:

- Purification of new PET-DLA materials was successfully performed in different media (solvents), including petroleum ether commonly used for purification of medical-grade polymers;
- The chemical composition of PET-DLA copolymer were unchanged after Soxhelet extraction
- The parameters of water extracts were comparable with thofor commercially available PCU

Acknowledgements

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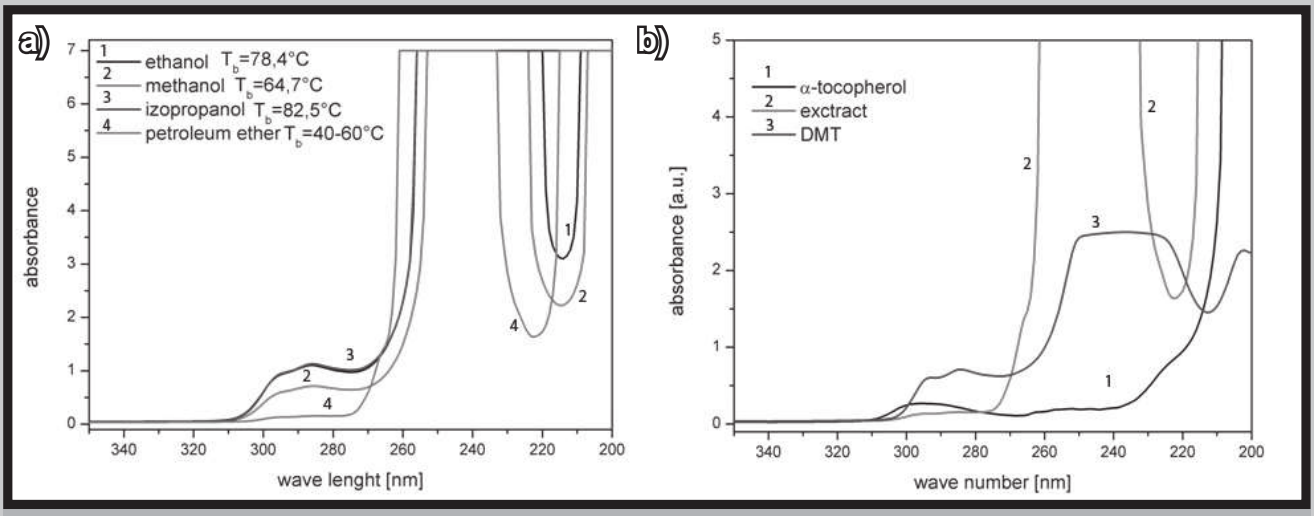

FIG. 2. UV-Vis spectra od different extracts (a) from PET-DLA polymer and the model solutions (b).

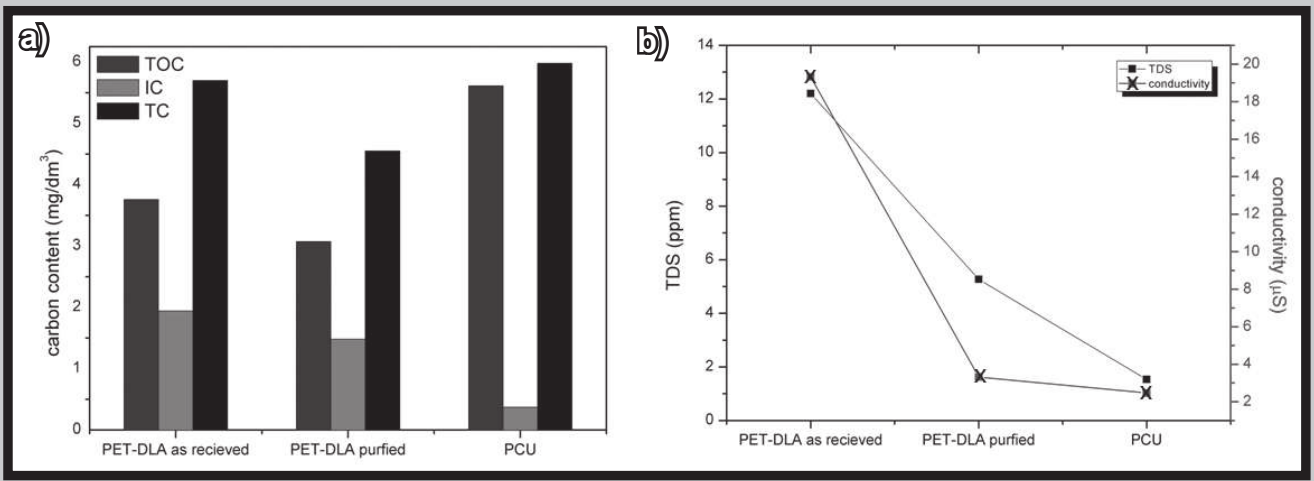

FIG. 3. The carbon content (a); TDS and conductivity (b) of water extracts from analysed materials.

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IN VITRO STUDY OF A NOVEL COLLAGEN - CALCIUM PHOSPHATE COMPOSITES

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Introduction

Reconstruction of bone defects lost due to trauma, cancer, or congenital defects is a major issue in orthopedic surgery [1]. Calcium phosphate (CaP) ceramics are widely used in bone regeneration. Excellent biocompatibility, bioactivity and biodegradability make CaP an ideal starting material for bone tissue engineering applications [2,3].

The aim of this work was to produce 3-D bioengineered composites of collagen and calcium phosphates (Col/CaP) by deposition of calcium phosphate within collagen matrix. The objective of the current study is the preliminary investigation of the in vitro cytotoxicity of a biomimetic collagen–calcium phosphate scaffold for orthopaedic.

Materials and methods

The high porous scaffolds were produced from a collagen solution using a freeze–drying technique. Collagen solutions with concentrations 2% (w/w) was prepared from lyophilized collagen in deionized water. Then, calcium phosphate formation in collagen scaffold was achieved. Collagen scaffolds were into a solution containing sodium ions for 3h, then were immersed into a calcium chloride solution for 3h. The next step were freezing and lyophilizing of scaffolds. After drying scaffolds were briefly washed in deionized water and freeze-dried.

Mouse fibroblast cell line 3T3 were seeded in the number of 1×10^6 cells/1 cm^2 and incubated for 7 days. After incubation, MTT assay was performed to assess the viability of 3T3 cells.

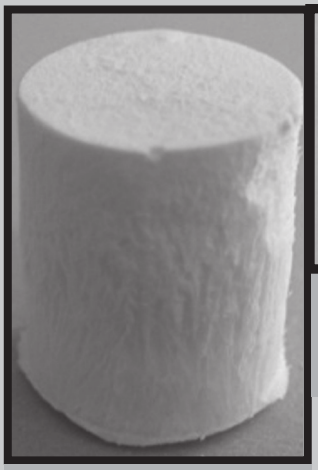

FIG. 1. Image of Col/CaP material.

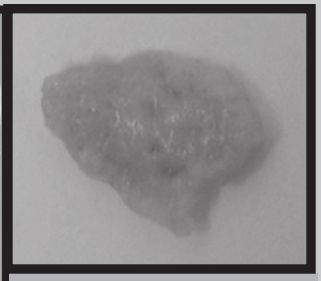

FIG. 2. Image of Col/CaP material after in vitro test and MTT assay.

Results

In FIG. 1. image of 3D Col/CaP material is presented and FIG. 2 shows a photograph of this material after in vitro testing.

The qualitative analysis of color intensity resulting from MTT assay showed that Col/CaP sample was quite well tolerated by the cells. As can be seen the cells were distributed only on the surface of the scaffold. Although the structure of the material was porous, the fibroblasts did not migrate into the material. The results from this study suggest, that calcium phosphate is precipitated primarily on the surface of the collagen matrix. Moreover, there may be a closing of pores in the material during the precipitation of the inorganic particles. These conditions are not conducive to cell adhesion and proliferation into collagen matrix.

Conclusion

In conclusion, our method allows to obtain 3D, porous Col/CaP materials. However, calcium phosphate was precipitated most of all on the surface of this material. Results concerning cell viability/proliferation evaluated by MTT assay showed viable cells on the surface of Col/CaP material.

Acknowledgements

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