STRUCTURE-PROPERTY CORRELATION STUDIES OF CROSS-LINKED CITRIC ACID-BASED ELASTOMERS

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Introduction

In recent years, poly(alkylene citrate) (PAC) biomaterials have become one of the most promising biomaterials for the production of scaffolds for soft tissue engineering [1]. Moreover, they were considered as components of numerous composites and copolymers studied in a wide range of possible medical applications. In terms of mechanical properties, PAC-based materials are similar to those of human tissues and can be readily tailored to specific needs [2] thus providing the opportunity of being adopted to a variety of bioengineering applications. In addition, cell proliferation studies indicated their remarkable biocompatibility and absence of cytotoxicity, with a significant decrease in thrombogenicity [3]. However, challenging structural characterization of PAC prepolymers has been reported in only a few reports despite the extensive published literature [4], with the majority of papers showing serious incoherencies in the discussion of the reported spectroscopic results. Hence, the main focus of this work was to optimize the composition and synthesis parameters of PAC polymers based on two diols: 1,6-hexanediol or 1,8-octanediol in two comonomer molar ratios, to obtain materials with defined structure and physicochemical properties. For evaluating their suitability as biomaterials, experiments were performed with adipose tissue-derived stem cells.

Materials and Methods

PAC prepolymers were synthesized in polycondensation reaction of citric acid and 1,8-octanediol (poly(octamethylene citrate) (POC)) or 1,6-hexanediol (poly(hexamethylene citrate) (PHC)) in a molar ratio of 2:3 or 1:1, at 140°C for 40 min, and later they were purified and freeze-dried. The prepolymers were characterized using 1D and 2D NMR techniques, mass spectrometry (ESI-MS) and acid value determination. Cross-linked PAC materials (cPAC) were obtained after postpolymerization of 30% ethanolic solutions of the prepolymers in specific molds (4 or 10 days at 80°C). The cPACs were characterized according to their mechanical properties. Evaluation of biological properties of the materials were conducted both on extracts of the materials and in direct contact using adipose tissue-derived stem cells.

Results and Discussion

NMR and MS results indicated the priority of the formation of linear oligomers, which directly translates into cross-linking density as well as mechanical and biological properties of the materials obtained. Our research also confirms the validity of using NMR analyses to determine the fundamental properties of cross-linked materials as early as at the stage of prepolymer synthesis. The values of tensile strength and maximum tensile strength at break of cPACs increase significantly with increasing cross-linking time while the reversed trend was observed for relative elongation and elongation at break. On the other hand, cPOC exhibit lower values of tensile strength and higher elasticity than cPHC materials which can be explained by the differences in chain length in the diol used for synthesis. The obtained results show differences in properties between the materials fabricated in 1:1 and 2:3 molar ratio in spite of significantly higher tensile strength values and decreased elongation for the latter materials. The first approach towards assessing cell behaviour in indirect contact with PAC polyesters was performed with the use of adipose tissue-derived stem cells (ASCs) (calcein-AM/propidium iodide counterstaining). Cells cultured within undiluted extracts were found dead or at the beginning of cell death, excluding cPHC_2:3_10d, where they were well spread. 5% extract from cPOC_1:1_10d also resulted to be fatal for ASCs. Their morphology and distribution on day 1 were well-defined and such parameters did not vary from cells cultured in control conditions. For *in vitro* test in a direct contact with ASCs, we selected cPHC_2:3_10d and cPOC_2:3_10d. On days 1 and 3, the cell number and morphology were similar on both studied polymers and on TCPS.

Conclusions

In this work, we presented structure-properties correlations of PAC biomaterials. Detailed NMR spectra combined with MS and acidity determinations allowed for a thorough understanding of the PAC structure, explanation of chemical nature of the synthesis process and shed a light on the chemistry of the material cross-linking process. The final and the most relevant conclusion of the presented paper is the correlation of the acidity, and the molar ratio of reactants with cell viability and proliferation studies results performed on material extracts. The results indicate the privilege of using a 2:3 molar ratio of reagents than commonly described in the literature 1:1 ratio while maintaining all the properties of the latter. In vitro tests performed in a direct contact with cells show that the final biological output can also be tuned by using diols of a higher number of carbon atoms in the chain.

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