ANTIOXIDANT ACTIVITY OF NOVEL PCL/BIOACTIVE GLASS COMPOSITES ENRICHED WITH POLYPHENOLIC COMPOUNDS EXTRACTED FROM FRUITS AND LEAVES OF SWEET CHERRY (*PRUNUS AVIUM* L.)

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Introduction

Biomaterials can cause an inflammatory response after implantation in living organism. Initial inflammation is an essential part of the tissue regeneration process since is required to modulate cell recruitment, differentiation and angiogenesis. However, excessive inflammation leads to formation of an adverse environment for regeneration and also to generation of reactive oxygen species (ROS). The ROS act as signalling molecules that upregulate cytokines and other inflammatory mediators that induce further inflammatory cell migration [1,2]. Therefore, biomaterials that can reduce the ROS production in a sustained and controlled manner and thereby modulate inflammatory response may be a useful tool for tissue engineering (TE) applications. An incorporation of anti-inflammatory agents into the biomaterial is a strategy for modulating inflammation [1]. Antioxidants are one of the most promising molecules that show anti-inflammatory effects by neutralizing ROS [3].

The aim of this study is to enrich poly(ε-caprolactone)/bioglass composite films with polyphenols (PPh) extracted from fruits and leaves of sweet cherry (*Prunus avium* L.) in order to combine the bioactive properties of bioglass with the biological activities and health benefits of polyphenols.

Materials and Methods

Conventional solvent extraction of polyphenols from fruits and leaves of sweet cherry was performed in 1,4-dioxane. Bioactive glass particles of the composition of (mol%) $40SiO_2$–$54CaO$–$6P_2O_5$ were synthesized by the use of sol-gel and melt-quenching methods. The polyphenols were introduced into materials directly with solvent-plant extract using solvent-casting method. The static water contact angle was evaluated by sessile drop technique. The amount of PPh present on the surface of materials was determined using Folin–Ciocalteu method. The antioxidant activity of the films was evaluated using DPPH, ABTS, and FRAP tests. Bioactivity of materials was assessed using SEM/EDX and FTIR methods after 7-day immersion in SBF.

Results and Discussion

The results showed that the entrapment efficiency of PPh derived from both fruits and leaves of sweet cherry was 100% for all materials, suggesting no loss of PPh and their activity. The content of PPh on the surface of materials containing leaf and fruit extracts increased in the following order: A2gel/PCL/PPh < A2melt/PCL/PPh < PCL/PPh (FIG. 1a). The reduction in the amount of PPh on the surface of composites can be explained by the adsorption of PPh on the surfaces of glass fillers.

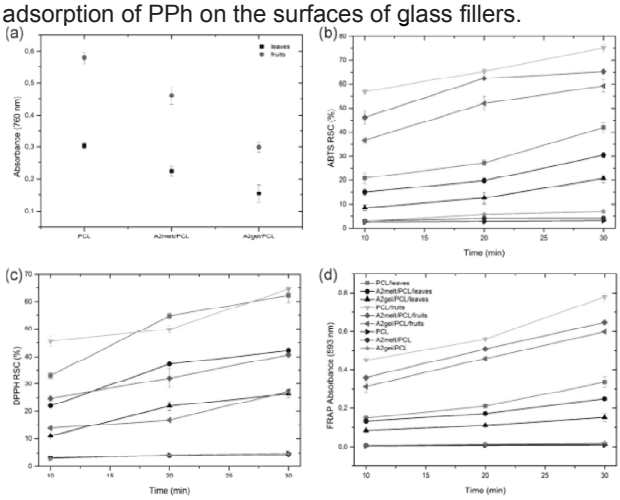

FIG. 1. The amount of PPh on the surface of materials (a), antioxidant activity of PPh-loaded films evaluated using ABTS (b), DPPH (c) and FRAP (d) assays. Results are expressed as mean ± SD (n=3).

After modification with PPh, the films exhibited significantly higher hydrophilicity that can be attributed to the exposition of hydroxyl groups of the PPh present on the surface of films. The antioxidant potential can be clearly ascribed to the presence of PPh in materials (FIGs. 1b-1d). PCL/PPh films showed the highest RSA and reducing potential, while the lowest values were found for A2gel/PCL/PPh materials. The results closely correlated with the content of PPh on the surface of films (FIG. 1a). The surfaces of all composites after soaking in SBF were fully covered with the thick layers rich in calcium and phosphorus. It seemed that layers formed on the films with PPh from fruits contained bigger and well-developed crystal forms. That could have been attributed to the higher concentrations of PPh on the surfaces of these composites. Phenolic hydroxyl groups can promote HCA deposition throughout the interaction with Ca^{2+} ions. The ATR-FTIR spectra of incubated materials were dominated by phosphate and carbonate bands (FIG. 3b) at 558 and 600 cm^{-1} (O–P–O bending mode), 1014 cm^{-1} with a shoulder at 1115 cm^{-1} (P–O stretching mode) and 875 cm^{-1} (CO_3^{2-} bending mode), characteristic for HCA layers.

Conclusions

Materials exhibited excellent *in vitro* bioactivity, improved hydrophilicity and also high antioxidant potential. The use of melt-derived or gel-derived bioactive glass particles and also PPh from leaves and fruits of sweet cherry, caused different PCL-PPh and BG-PPh interactions and therefore affected PPh content on the film surfaces. That, in turn, determined surface wettability, *in vitro* bioactivity, and finally antioxidant activity, providing possibilities for modulating these properties in a wide range. The results suggest that obtained films represent a potential multifunctional biomaterials for bone tissue regeneration.

Acknowledgments

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