BACTERIAL CELLULOSE/POLYHYDROXYALKANOATES COMPOSITES: OBTAINING, CHARACTERIZATION AND EVALUATION OF BIOCOMPATIBILITY

P. Stanescu[a*], C. Zaharia[a], B. Galateanu[b], E. Vasile[a], A. Casarica[c]

[a] *Advanced Polymer Materials Group, Polytehnic University of Bucharest, 149 Calea Victoriei, Bucharest 010072, Romania*
[b] *Department of Biochemistry and Molecular Biology, University of Bucharest, Romania*
[c] *National Institute for Chemical Pharmaceutical Research and Development, Bucharest, Romania*
**paul_stanescu@yahoo.com*

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Abstract

The research study focuses on the preparation and characterization of composite materials based on bacterial cellulose and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) as biodegradable scaffolds for tissue engineering applications. Bacterial cellulose/polyhydroxyalkanoates composite membranes were prepared from BC membranes and PHBHV chloroform solutions. Dried bacterial cellulose membranes were immersed in PHBHV solutions for 24 h and the composite materials obtained were left to dry at room temperature for 24 h. For comparison PHBHV films were prepared. Macroscopically homogeneous composite membranes were characterized by scanning electron microscopy, Fourier-transformed infrared spectroscopy and X-ray diffraction. Composites biocompatibility was evaluated by cell attachment studies on human adipose derived stem cells (hASCs). Biological investigations showed that most of the cells retained their typical morphology with extensions proving that the composite materials did not exhibit cytotoxic effect. Interestingly, as revealed by the fluorescence visualisation of the living cells, hASCs on BC/PHBHV composites displayed an organized orientation as compared to the random patterns observed in BC and PHBHV, respectively the control.

1. Introduction

Cellulose is a linear polymer made of glucose molecules linked by β(1–4) glycosidic linkages. It has traditionally been sourced from plants. However, refining of plant cellulose typically involves harsh, aggressive processing to remove non-cellulose materials such as lignin and hemi-cellulose. Fortunately, bacterial cellulose (BC) represents an alternative source of cellulose where no chemical or mechanical refining is necessary [1-3]. The main production is based on the biosynthesis of cellulose by different microorganisms, including bacteria, algae and fungi. BC differs from plant cellulose with respect to its purity, high crystallinity, ultrafine network structure, high water absorption capacity, high mechanical strength in the wet state, availability in an initial wet state and biocompatibility [3]. Owing to its biocompatibility, BC has also recently attracted a great deal of attention for biomedical

applications: artificial skin for burn or wound healing material, artificial blood vessels for microsurgery. The potential of BC scaffold for *in vitro* and *in vivo* tissue regeneration also continues to be explored and shows great promise [4-5].

Polyhydroxyalkanoates (PHAs) are a class of natural thermoplastic polymers with similar properties to those of conventional plastics. They have attracted much interest as alternatives to synthetic polymers, the more so as they can be produced from renewable resources and processed with the aid of equipment used for polyolefins or other synthetic materials [6-7]. As these biopolymers are biodegradable and biocompatible, they are suitable for many biomedical applications such as surgical sutures, long term carriers of drugs and tissue engineering. Poly(hydroxybutyrate-co-hydroxyvalerate) (PHBHV) is a bacterially derived co-polymer produced by fermentation. Belonging to the family of polyhydroxyalkanoates, this biopolymer has been the focus of several studies investigating its thermal, physical and mechanical properties and its use in scaffolds for tissue engineering.

To broaden the biomedical applications of BC, various attempts have been made to produce BC composites with high functionality. Among them, BC/PHAs composite is one of candidates that have great potential applications for tissue engineering [8].

This paper work focuses on the preparation and characterization of composite materials based on bacterial cellulose and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) as biodegradable scaffolds for tissue engineering applications (blood vessel engineering).

2. Materials and methods

2.1. Materials

Bacterial cellulose membranes were kindly provided by National Institute for Chemical Pharmaceutical Research and Development (ICCF Bucharest, Romania). The bacterium used in all the experiments for obtaining BC was *Acetobacter xylinium DSMZ* (ICCF 398). Poly(3-hydroxybutyrate-co-3-hydroxyvalerate2%) (PHBHV, Good-Fellow) copolymer was gently dissolved in boiling chloroform under stirring for 12 h (5 wt. %).

Dried bacterial cellulose membranes were immersed in polyester solutions for 24 h and the composite materials obtained were left to dry at room temperature for 24 h followed. For comparison PHBHV films were prepared by casting from chloroform solutions on Petri glass plates.

2.2. *Methods*

FT-IR spectra were taken on a Jasco 4200 spectrometer equipped with a Specac Golden Gate attenuated total reflectance (ATR) accessory, using a resolution of 4 cm−1 and an accumulation of 60 spectra, in the 4000–600 cm−1 wavenumber region. XRD patterns were obtained using a RIGAKU miniflex II diffractometer with CuKα radiation. Morphological information of the composites samples was obtained through the scanning electron microscopy analysis of the gold-coated specimens. The analysis has been performed using a QUANTA INSPECT F SEM device equipped with a field emission gun (FEG) with a resolution of 1.2 nm and with an X-ray energy dispersive spectrometer (EDS)

Cell culture and cytotoxicity test on silk hydrogels

Human adipose derived stem cells (hASCs) were seeded at an initial density of 1.5×10^4 cells/cm^2 on the composites surfaces and maintained in standard conditions of culture for one week. The viability of hADSCs in contact with the novel composite materials was

assessed at 48h post seeding using qualitative Live/Dead Assay. Cell viability on the novel composites materials was evaluated by fluorescencemicroscopy using Live/Dead Kit (Invitrogen, Life Technologies, Foster City, CA, USA).

3. Results and discussions

Macroscopically relatively homogenous composite membranes were obtained. Pure swollen bacterial cellulose and BC/PHBHV composites appear transparent milky and opaque white respectively. SEM microphotographs clearly reveal the BC nanofribrils covered or embedded with polyhydroxyalkanoate particles (fig.2)

Fig.1. Images showing swollen BC (left) and BC/PHBHV composites after liophyllization (right)

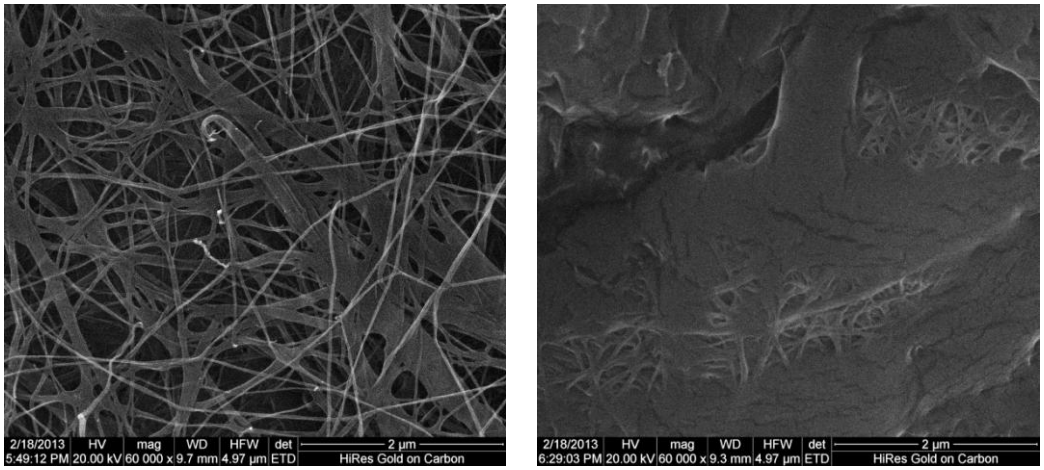

Fig.2. SEM microphotographs showing the fine nano-fibrillar morhology of pure BC (left) and composite materials based on BC/PHBHV in which the copolyester covers the BC nanofibrils (rigth)

The ATR-FTIR spectrum of bacterial cellulose revealed the presence of characteristic peaks for cellulose at 3338 cm^{-1} (OH), 2970 and 2895 cm^{-1} (CH_2), 1371 cm^{-1} (CH), 1158 cm^{-1} (C-O-C), 1105 cm^{-1} (C-C), 1052 cm^{-1} (C-O), figure 2. In the spectrum of composite an intense peak at 1725 cm^{-1} appears and it corresponds to specific vibration of C=O from PHBHV (spectra not shown).

The high degree of crystallinity of cellulose is evidenced by 3 diffraction peaks for bacterial cellulose at 2θ diffraction angles of 14.5°, 17° and 22.76°. Diffraction patterns for composite membranes show mainly the bacterial cellulose peaks at 1/1 ratio between

BC/PHBHV (fig.3). When the ratio is in favour of PHBHV the peaks characteristic for polyhydroxyalkanoates could be seen on the diffractograms.

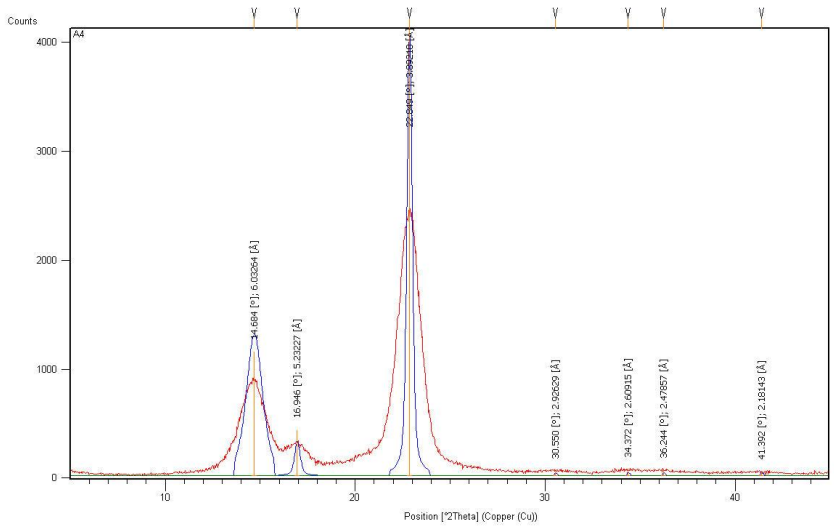

Fig.3. XRD diffractogram for BC/ PHBHV composite 1/1 ratio

In order to examine cell survival up to one week, the viability of hADSCs in contact BC, PHBHV and BC/PHBHV composite samples was evaluated at 48h post seeding using Live/Dead assay. Interestingly, as revealed by the fluorescence visualisation of the living cells, hASCs on composite specimens displayed an organised orientation as compared to the random patterns observed in all other samples, including the control. This observation could highlight new possible biomaterials candidates for angiogenic differentiation support of hASCs in the view of vessel engineering.

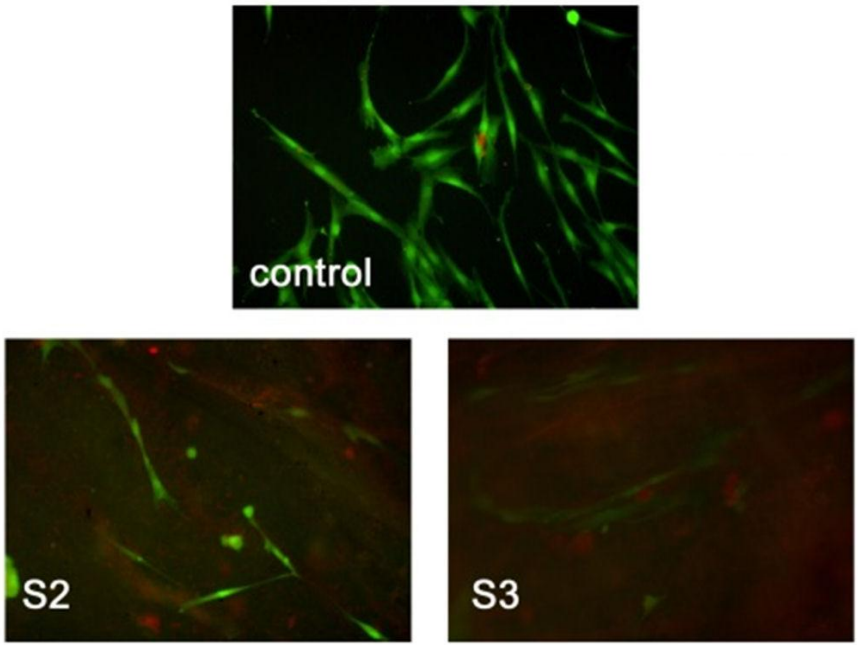

Fig.4. Fluorescence microscopy detection of live (green labeled) and dead (red labeled) human adipose derived stem cells in contact with control, BC/PHBHV, 1/6 w/w S2 and BC/PHBHV, 1/4 w/w S3 composite materials at 48h

4. Conclusions

BC/PHBHV composite materials were developed by immersion of bacterial cellulose membranes in chloroform copolyester solutions (various mass ratios). Valuable biological results from this study highlight new possible biomaterial candidates for angiogenic differentiation support of hADSCs in the view of blood-vessel engineering application. Such composite material based on natural polymers could ensure the required mechanical integrity of the scaffold and controllable biodegradability.

Acknowledgements:

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