POLYHYDROXYALKANOATE BIOPOLYMERS FOR MEDICAL APPLICATIONS

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Introduction

Biopolymers are promising materials for tissue engineering [1]. One of such materials is a group of polyesters synthetised by microorganisms, namely polyhydroxyalkanoates (PHA). These biomaterials are completely biocompatible with mammalian tissues, which makes them appropriate for tissue engineering purposes. Depending on the (*R*)-3-hydroxyacid that constituents the polymer chain, the PHAs tend to vary in its physicochemical and mechanical properties (i.e., brittle, hard to elastic), offering a wide range of potential applications. Thanks to the broad features, these polyesters can be formed into purpose-specific materials through commonly used procedures. Moreover, PHAs can serve as a platform for controlled drug release which occurs locally only in the site of an implantation. Here, we present a complete route to obtainment and characterisation of two types of PHAs used for construction of wound patches.

Materials and Methods

Material synthesis Through microbial fermentation two PHA polymers were synthetised in 5L fermenters. *Z. denitrificans* converted glycerol to polyhydroxybutyrate (PHB), whereas *P. putida* converted octanoic acid to polyhydroxyoctanoate (PHO). PHAs were extracted form dried biomass, purified over charcoal, precipitated in methanol and resuspended in $CHCl_3$. Three methods were used to fabricate wound patches: electrospinning, wet-spinning and solvent casting-porogen (NaCl) leaching to water (SCPL). The materials were characterised by SEM or μ-CT. PHO was also modified with PHO-diclofenac-oligomers obtained by p-toluenosulphonic acid mediated synthesis [2]. *Biological characterisation* Mouse fibroblast cells were used for primary assessment of PHO-derived materials. Firstly, the cytoskeleton structures (actin, microtubule, vimentin, nuclei) were stained and observed using confocal microscopy in order to assess cell attachment and material impact on the cell morphology [3,4]. Further, migration studies were conducted, which were followed by *in vitro* wound healing assays [4]. Lastly, the SCPL patches (non and modified with diclofenac) were used in mouse *in vivo* model wound healing assay. Mice were sampled at weekly intervals (7, 14 days) and healing progress was assessed by three methods: immunohistochemically (IHC), by assessing the genome at the mRNA level by Real Time PCR (qPCR) and testing the interleukin level (IL) by enzyme immunoassay - ELISA (E).

Results and Discussion

Fermentation processes led to accumulation of two types of polymers. PHB was accumulated within 48 hours, biomass reached 96 g L^{-1} with 28% polymer content; whereas PHO process lasted for 30 hours and system accumulated 122 g L^{-1} of bacteria of which 71% was polymer. The PHAs were used to create three distinct structures (FIG. 1).

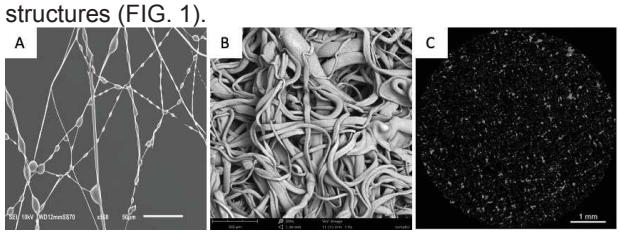

FIG. 1. Wound patches materials obtained by electrospinning PHO (A), wet spinning PHB (B) and SCPL method (C).

PHO was chosen for further studies. At first cellular morphology was observed of cells grown on flat PHO films. There were much more cytoskeletal bifurcations on the polymer than on glass, which suggests that PHO favours cell attachment. Next, cell migration studies revealed non-directional movement across the PHO surface, further confirming the biocompatibility of the PHA polymer. The *in vitro* wound assay allowed to conclude that PHO favours artificial wound closure, which is faster when compared to a polylactic acid control. Lastly, the synthetised PHO-diclofenac-oligomers were blended with PHO polymer, and its cytotoxicity was assessed. Based on the above preliminary studies two types of SCPL sponges were prepared: PHO-based and modified PHO with oligomers (100 μg/1g), which were tested in mouse model for wound healing capabilities. Application of either patch allowed for partial wound closure with material integration into the wound. Cells were migrating to the patches creating new blood vessels. There was low pro-inflammatory cytokine concentration and $CD68^+$ macrophages level, thus leading to the conclusion that materials are beneficial for the process of wound healing *in vivo*.

Conclusions

Fermentation process allows to obtain a wide range of PHA polymers, which can serve as substates for cell attachment and growth. PHAs can be easily turned into biomaterials with desired properties by commonly used methods (i.e. electrospinning, wet spinning or SCPL). On the example of PHO, it was proved that it is a promising material for wound healing process both by *in vitro* and *in vivo* studies. Further, PHO, being chemically pliable, can serve as a platform for local drug release.

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