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Designing exopolysaccharide-graft-poly(3-hydroxyalkanoate) copolymers for electrospun scaffolds

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ABSTRACT

Poly(3-hydroxyalkanoate)s PHAs have been demonstrated to be a family of biopolyester with good biodegradability and biocompatibility. A glycosaminoglycan-like marine exopolysaccharide EPS HE800 was here incorporated to enhance cell adhesion. Novel graft copolymer HE800-*g*-PHA were prepared to improve the compatibility between hydrophobic PHA and hydrophilic HE800. The carboxylic end groups of PHA oligomers were activated with acyl chloride functions, allowing coupling to hydroxyl groups of HE800. Fibrous scaffolds were prepared by a modified electrospinning system which combined simultaneously PHA electrospinning and HE800-*g*-PHA copolymer electrospraying. Adhesion and growth of human mesenchymal stem cells on the HE800-*g*-PHA scaffolds showed a notable improvement over those on PHAs matrices.

1. Introduction

Poly(3-hydroxyalkanoates) (PHAs) are a class of natural biodegradable polyesters accumulated by many bacteria as carbon and energy supply when an essential nutrient is limited [1,2]. Using various substrates, a wide variety of PHAs are synthesized, differing notably by the length of their side chains [3]. Two types are distinguished, short chain length PHAs, or *scl*-PHAs, possessing alkyl side chains having up to two carbon atoms, for example the widely used poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBHV; medium chain length PHAs, or mcl-PHAs, with at least three carbon atoms on their side chains. The length of side chains leads the physical properties of PHAs, crystalline, rigid and brittle for *scl*-PHAs, semi-crystalline, soft and elastomeric for the mcl-PHAs. Thanks to their biocompatibility, PHAs proved to be good candidates for biomedical applications including biomedical devices, biodegradable drug carriers or tissue engineering (TE) [4–6]. Fibrous scaffolds obtained from electrospun biodegradable PHAs have attracted widespread attention because of their nanostructure morphology which can mimic the extracellular matrix component (ECM) [7,8]. Despite its many attributes, the intrinsic hydrophobic properties of PHAs restrict their applications as cell-colonizing materials [9]. One approach to improve its biological properties can be made through blending PHA with hydrophilic polymers and compounds [10,11]. Among polymers used in TE, polysaccharides have been widely studied due to their biodegradability, biocompatibility and capacity to mimic the extracellular matrix [12–17]. Here we proposed to test a marine exopolysaccharide (EPS), HE800 which is a hyaluronane-like polysaccharide, secreted from marine bacteria *Vibrio diabolicus* originating from deep sea hydrothermal vents. Its large scale production is therefore convenient, low cost, reproducible and free of non-conventional transmissible agent. HE800 is a non-sulfated linear polysaccharide. Its repeating unit is a tetrasaccharide composed of two glucuronic acid units, one N-acetyl-glucosamine and one N-acetylgalactosamine (see Scheme 1) [18,19]. The presence of carboxylate groups on HE800 make this polysaccharide promising candidates to enhance interactions with proteins such as growth factors and cytokines. In a previous study, fibers of HE800 were used to fill critical size defects in rat calvaria and showed strong bone healing effect after three weeks [20]. HE800 also induced the attachment of chondrocytes on a Si-hydroxypropylmethylcellulose-based hydrogel [19].

In this context and as a preliminary study to further TE applications, we tested the ability to support cell proliferation of a scaffold based on PHA and HE800. The PHA used in this study was the PHBHV as it is more convenient for electrospinning process than

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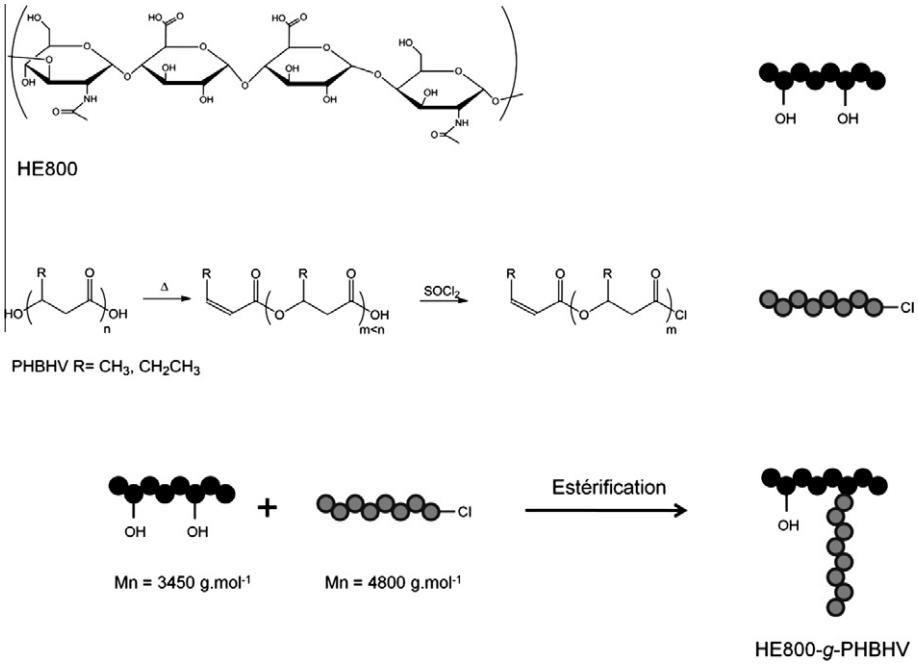


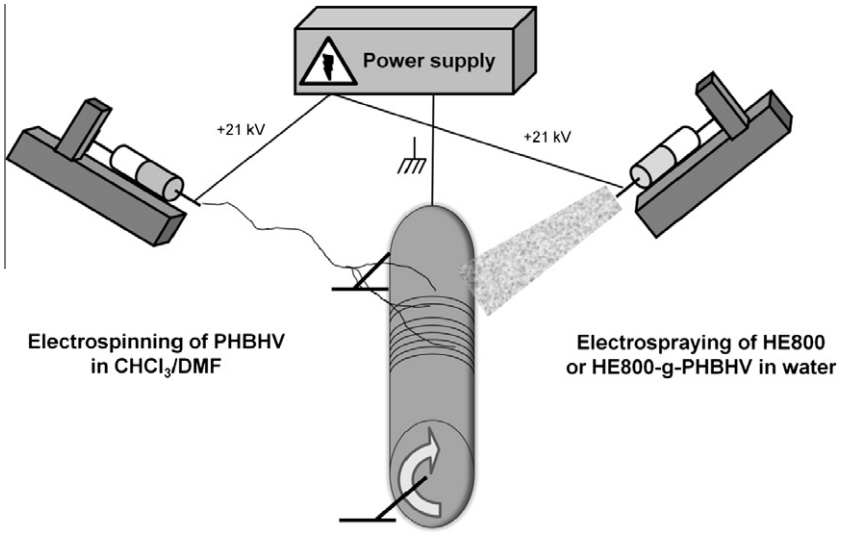
Scheme 1. Preparation of the copolymer HE800-*g*-PHBHV.

the homopolymer PHB. We first prepared a graft copolymer HE800-*g*-PHBHV to improve the compatibility between hydrophobic PHBHV and hydrophilic HE800. In the grafting onto method, the carboxylic functions of PHBHV were activated with acyl chloride functions, allowing coupling to hydroxyl groups of HE800. The chosen method for the preparation of the scaffold is a modified electrospinning system which combined both electrospinning and electrospraying processes (Scheme 2) previously described by Ekaputra et al. [21]. PHBHV was electrospun and the HE800-*g*-PHBHV was simultaneously electrosprayed onto the PHBHV fibers. Furthermore, we studied the ability of the scaffold based on the electrospun PHBHV, $PHBHV_{e\text{-}spun}$, fibers combined to electrosprayed HE800-*g*-PHA, HE800-*g*-$PHBHV_{e\text{-}sprayed}$, to support human mesenchymal stem cells (hMSCs) adhesion and proliferation.

2. Materials and methods

2.1. Materials

PHBHV with 12% of hydroxyvalerate was purchased from goodfellow and was purified by precipitation in ethanol. The exopolysaccharide HE800 (M_n of 3450 g mol^{-1}), obtained from LBMM of Ifremer (France) was dried overnight under vacuum at 100 °C before use. DMF (analytical grade), chloroform (analytical grade), dichloromethane (analytical grade) and ethanol (analytical grade) were purchased from SDS and used without further purification. Chlorobenzene (99.5%), thionyle chloride, anhydrous dimethylsulfoxyde (DMSO) (99.99%), hexamethyldisilazane (HMDS) and glutaraldehyde were purchased from Sigma–Aldrich. Dialysis



Scheme 2. Schematic illustration of the co-electrospinning–electrospraying process.