

BIODEGRADABLE BICOMPONENT FIBERS FROM RENEWABLE RESOURCES

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ABSTRACT

There is a strong need for medical textiles that have appropriate mechanical properties for a given time but should be completely resorbed after a given period. This study was focused on polyesters from renewable resources, namely poly(3-hydroxybutyrate) (PHB), its copolymer with 3-hydroxyvalerate (PHBV) and the FDA-approved polylactide (PLA). Melt spinning experiments demonstrated that monofibers could be produced only using PLA. Because of significant acid formation of PLA fibers, a bicomponent approach was followed. The resulting bicofiber with PHBV and PLA as core and sheath material, respectively, showed suitable properties and good *in vitro* biocompatibility in presence of human dermal fibroblasts.

Key Words: polyhydroxyalkanoate, phb, pla, bicomponent fiber, biocompatibility

1. INTRODUCTION

Tailored biopolymer fibers play an important role in agriculture, filtration, packaging, hygiene and protective clothing, as well as in the medical field (drug delivery systems, wound closure and healing products, surgical implant devices) [1]. A biodegradable implant will not necessitate a second surgical event for removal [2]. It can be engineered to degrade at a rate that will slowly transfer load to a healing tissue and/or as the basis for a drug delivery system [3].

All polyesters degrade eventually, with hydrolysis being the dominant mechanism. While aromatic polyesters such as PET exhibit excellent material properties, they prove to be almost totally resistant to microbial attack. Aliphatic polyesters on the other hand are readily biodegradable, but usually lack good processability and mechanical performance due to low molecular weight [4, 5].

Most commercially available biodegradable polyesters are petroleum-based. To avoid perturbation of the ecosystem, biopolymers should be derived from renewable resources instead of oil reserves and their production needs to be performed in an environmentally friendly way [6]. Polyhydroxyalkanoate (PHA) is produced naturally and polylactide (PLA) is synthesized from renewable resources [4].

PLA is regarded as renewable plastic since its raw material, lactic acid, is generally produced by bacterial fermentation from corn starch or sugar cane [7, 8]. PLA degrades by hydrolysis and enzymatic activity and has a range of mechanical and physical properties that can be engineered appropriately to suit a particular application [2]. The melt spinning of pure PLA from different sources has been studied extensively [9, 10].

Polyhydroxyalkanoate (PHA) is a linear polyester accumulated by microorganisms as an intracellular reserve of carbon and energy [5, 6]. Among the PHAs, poly(3-hydroxybutyrate) (PHB) is the most studied and the easiest to produce [11]. The most common commercial PHA consists of a copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) [4].

Thermal degradation and the complex crystallization behavior render melt spinning of PHB fibers rather problematic [12, 13].

Bicomponent fibers are considered to be one of the most interesting developments in the field of synthetic fibers [14]. Two molten polymers can be merged just before a spinneret capillary, so that the single filaments of the finished yarn consist of two joined components [15]. Empa's custom-made pilot melt-spinning plant allows producing mono- and bicomponent fibers with various fiber cross-sections and material combinations with a throughput of up to several kg h⁻¹ [16, 17].

The goal of this work is to develop biodegradable fibers from renewable sources that show best performance with respect to mechanical properties and colonization of tissue engineered cells.

2. MATERIAL AND METHODS

2.1 Biomaterials

Poly(3-hydroxybutyrate) (PHB) was obtained from Biocycle (Biocycle 1000, Biocycle, Brasil). Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with a 3-hydroxyvalerate content of 8 mol% was bought from Tianan (Enmat Y1000, Tianan Biologic Material, China). Polylactide (PLA) was obtained from NatureWorks (PLA 6200D, NatureWorks, U.S.A.).

Table 1. Physical properties of polyesters used in this study. Mw: molecular weight average; PDI: polydispersity index; Tm: melting temperature; Tg: glass transition temperature.

| Polyester | Mw [kDa] | PDI [] | Tm [°C] | Tg [°C] |
|-----------|-------------|------------|------------|------------|
| PHB | 556 | 3.8 | 176 | 5.9 |
| PHBV | 260 | 3.2 | 165 | 5.1 |
| PLA | 109 | 2.1 | 160 - 170 | 60 - 65 |

2.2 Spinning of Bicomponent Fibers

The fiber melt spinning was carried out on Empa's custom-made pilot melt-spinning plant built by Fourné Polymertechnik (Alfter-Impekoven, Germany) to our specifications. The bicomponent plant enables the prototype production of mono- and bicomponent fibers with various fiber cross-sections and material combinations with a throughput of up to several kilograms per hour [17]. Monofilaments in the range of 50 - 100 dtex can be produced.

2.3 Tensile Testing

The load-strain behavior of the fibers was tested using a tensile tester Tensorapid 3 by Uster Technologies (Uster, Switzerland), equipped with a 500 N load cell. Single filament tests with 100 mm test length and a constant rate of extension of 100 mm/min were performed.

2.4 Knitting of Fibers

To prove processability and to provide substrates for *in vitro* and *in vivo* tests, samples were knitted from the spun fibers. For this purpose we used a single circular knitting machine Elha by Harry Lucas Textilmaschinen (Neumünster, Germany) to produce single jersey fabrics. The head diameter was 102 mm, the needle gauge 1.6 mm.

2.5 Biocompatibility

For biocompatibility tests three layers of textile samples were fixed in a Cell Crown Insert (Scaffdex) to keep the textiles stretched and on the bottom of a tissue culture 6-well plate. Textiles samples were sterilised with 70% ethanol for 1 h and afterwards air dried.

Human dermal fibroblasts were cultivated in DMEM, 10%FCS, 1%PSN (penicillin-streptomycin-neomycin antibiotic mixture) in 5% CO₂ and aggregates of cells were used to seed the textiles. A cell suspension of 200.000 fibroblasts was centrifuged in a 15 ml tube (13 min, 60 x g). The obtained pellet and the supernatant were incubated at 37°C for 5h to allow cell reaggregation to occur. In preparation for cell seeding 4 ml of cell culture media was added to the textiles, allowed to equilibrate for 30 minutes and the cell aggregates were directly applied on top of the textiles in approximately 0.5 ml of medium.

After 11 days with medium changes every three days the cells were fixed for immunohistochemical triple staining of actin, vinculin and nuclei. Cells were washed three times with phosphate buffered saline (PBS) and treated with 4% paraformaldehyde plus 0.2% Triton-X for 8 min. The cells were then washed again with PBS. Actin filaments were stained with Alexa Fluor 488 phalloidin (0.165 μ M, Invitrogen, Molecular Probes) in PBS for 20 min. Vinculin protein was stained by h-Vin-1 monoclonal anti-human vinculin (1:300, mouse IgG1, Sigma) in PBS with 1.5% dried milk powder for 2 h and the second antibody Alexa Fluor 546 labelled goat anti-mouse (IgG) (1:400, Invitrogen, Molecular Probes) in PBS with 1.5% dried milk powder for 2 h. Cell nuclei were finally stained with DAPI (4'-6-Diamidino-2-phenylindole, 10 μ g/ml, Sigma) in PBS for 1h. Triple stained cells were imaged using the fluorescence microscope Axio Vision (Zeiss).

3. RESULTS AND DISCUSSION

PHB with a molecular weight of approx. 500 kDa could not be drawn to a fiber. Because of thermal degradation the material was too brittle after extrusion, spinning and quenching. The crystallization rate of PHB is known to be very low and as a consequence large spherulitic structures develop which cause poor mechanical properties [18]. Cracks between spherulites and secondary crystallization of the amorphous phase during storage cause brittleness [19]. Incomplete crystallization leads to adhesion of the single fibers of a multifilament yarn, which increases with storage time [20].

3-hydroxyvalerate (HV) units disrupt the crystallites - the higher the percentage of HV units in the polymer the lower is the amount of crystals. Thus a compromise between low melt temperature and enough crystallinity has to be found [21]. PHBV, with a HV content of 8

mol%, showed better melt stability due to a lower melting temperature. Nevertheless, a pure PHBV fiber could not be produced due to winding problems (stickiness of the fiber).

Temperature, residence time and shear forces have a significant influence on the molecular weight degradation of biopolymers that corrupts processability [22]. To overcome this problem a high initial molecular weight is favored. Yet a highly viscous polymer asks for high extrusion temperatures that promote degradation. A PHBV copolymer with different HV content was produced at Empa [23]. Due to its high molecular weight of 1,600,000 Da the processing temperature had to be kept at a temperature of ca. 180°C where the degradation of the polymer is critical. Thus no fiber could be produced.

Possible reasons for failing to produce a fiber from PHB or PHBV were too low initial molecular weights, critical polymer degradation by hydrolysis and/or shear degradation, as well as hindered crystallization due to short dwell time in the quenching chamber. The addition of a nucleating agent (e.g. talc, cyclodextrin) did not improve the performance.

On the other hand, PLA could be spun to fibers with reasonable mechanical properties. To overcome the difficulties with spinning PHB, bicomponent fibers with a PHBV core and a PLA sheath were successfully spun. The bicomponent fibers with PLA as a sheath material were strong enough (see Figure 1) for a successful construction of a textile fabric.

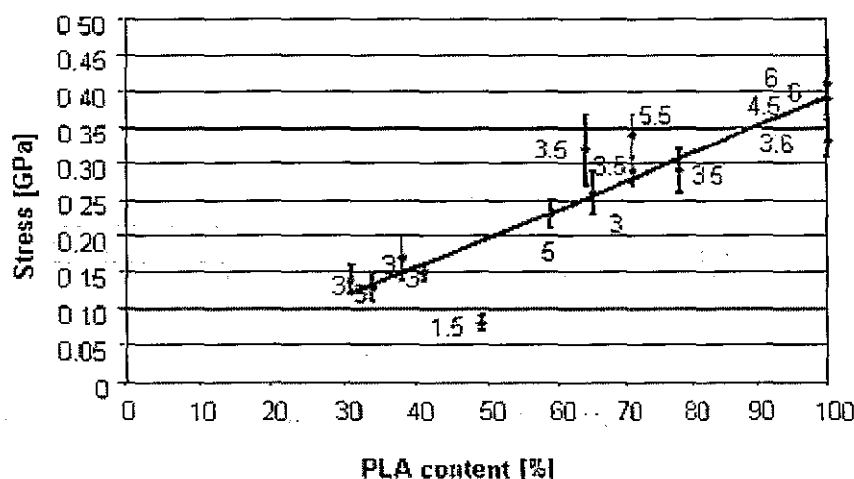


Figure 1. Tensile strength of bi-component fibers with PHB as core and PLA as sheath material as a function of the weight percentage of PLA content. Numbers next to data points indicate draw ratio of the fiber. Error bars were calculated based on 6 tensile measurements.

Abiotic (sterile) degradation tests with bi-component fibers with PLA as the sheath material in isotonic salt solution showed no significant loss of molecular weight and tensile strain but a reduction of the tensile stress (5-33%) over a month of incubation. Such tests also showed a drop of the pH to as low as 4.7 in non-buffered, isotonic salt solution which is expected to correlate with the release of lactic acids upon degradation of the material.

In vitro biocompatibility studies with human dermal fibroblasts showed no toxicity of the bicomponent fibers despite the presumed production of acidic lactic acid under cultivation conditions. Fibroblasts growing out from cell reaggregates adhered to the textiles and grew

along single fibers, covering them well after a cultivation period of 1 week making them to good candidates for medical therapeutic approaches.

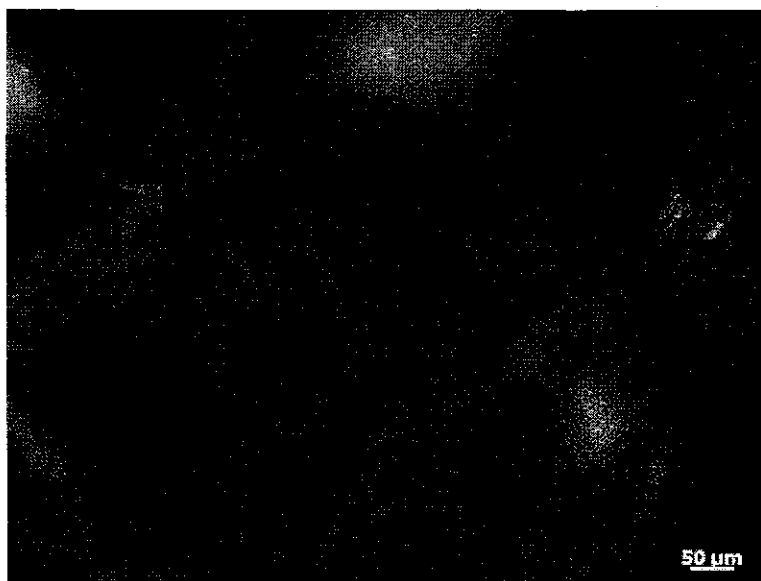


Figure 2. Cell aggregates of normal human dermal fibroblasts NHDF were applied on top of a bi-component fiber of PHBV and PLA (core and sheath). After 11 days the cells were fixed for triple histochemical staining. Cell attachment and proliferation from reaggregates were imaged using fluorescence microscopy. Cell nuclei: blue; actin (cytoskeleton): green; vinculin (focal adhesions): red

4. REFERENCES

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