

## Medium-chain-length polyhydroxyalkanoate: a bacterial biopolyester for medical applications?

P. Furrer<sup>1,3</sup>, K. Maniura<sup>2</sup>, S. Zeller<sup>1</sup>, S. Panke<sup>3</sup> and M. Zinn<sup>1</sup>

<sup>1</sup>Laboratory for Biomaterials, <sup>2</sup>Laboratory for Materials Biology Interactions, Materials Science and Technology, EMPA, Lerchenfeldstr. 5, 9014 St. Gallen, Switzerland

<sup>3</sup>Swiss Federal Institute of Technology Zurich ETHZ, Institute of Process Engineering, Bioprocess Laboratory, Universitätsstr. 6, 8092 Zurich, Switzerland

**INTRODUCTION:** Medium-chain-length poly([R]-3-hydroxyalkanoate) (mclPHA, monomers from C<sub>6</sub>-C<sub>14</sub>) is a water insoluble, biodegradable, and biocompatible class of biopolyesters of microbial origin. Its use for medical applications has been proposed<sup>1</sup> but due to the limited availability only a few studies have been carried out<sup>2</sup>.

A delicate point is the possible contamination of mclPHA with endotoxins due to its production in Gram negative bacteria. To date, chlorinated solvents have been used to extract mclPHA from freeze-dried biomass. A novel method has been developed that allowed extraction and reduction of contaminations using green chemistry.

In this work the interaction between mclPHAs and 3T3 fibroblasts or human bone marrow cells (HBMCs) was studied. The attention was turned to cell adhesion and polymer degradation.

**METHODS:** Three different mclPHAs were produced in *Pseudomonas putida* GPo1 and extracted using non-halogenated solvents. The monomer composition of the Poly(3-hydroxyoctanoate-co-3-hydroxy-10-undecenoates) was 100/0 w% (PHOU(100)), 50/50 w% (PHOU(50)) and 0/100 w% (PHOU(0)). The endotoxicity was determined by the limulus amebocyte lysate test and was below 10 EU/g mclPHA. The polymers were dissolved in methylene chloride (3 w%) and cast to glass platelets. The solvent was evaporated at room temperature for 2 days. The polymer films were dried at 80°C for 1 hour.

The coated platelets were placed in 6-well plates and about 20'000 fibroblasts or HBMCs were seeded on the polymer films in proliferation medium. They were studied after 3 and 10 days under the fluorescence microscope and eventual weight losses caused by degradation were determined gravimetrically after removing the cells. Contact angles were measured with a Krüss contact angle measuring system G10.

**RESULTS:** Good biocompatibility could be observed for 3T3 fibroblasts and for HBMCs on

the 3 PHOUs. 3T3 fibroblasts adhered well on all mclPHA films. The proliferation of HBMCs on PHOU(100) was good and the distribution uniform as shown in Figure 1. Interestingly, on PHOU(50) and PHOU(0) aggregate formation could be observed. After 10 days, no polymer loss could be measured gravimetrically.

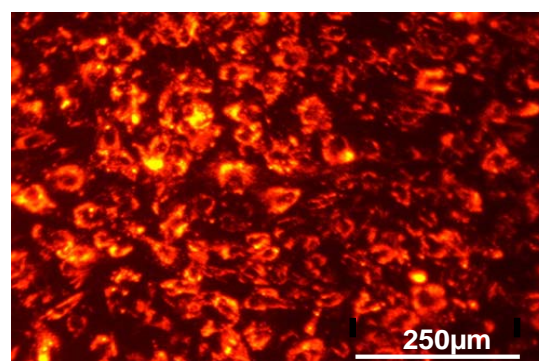


Figure 1: Fluorescently stained HBMCs on PHOU(100) after 10 days of proliferation.

Table 1. Molecular weights and water contact angles of mclPHAs.

MclPHA	M <sub>w</sub> [kDa]	Water contact angle [°]
PHOU(100)	236	98 ± 5
PHOU(50)	285	109 ± 5
PHOU(0)	304	113 ± 5

**DISCUSSION & CONCLUSIONS:** The experiments show that PHOU are biocompatible to 3T3 fibroblasts and HBMCs. 3T3 fibroblasts and HBMCs proliferated well on all PHOUs although their surfaces were hydrophobic as shown in Table 1. Biodegradation of the high molecular weight PHOUs is most likely slow so that no weight loss could be measured within 10 days. Further experiments are needed to elucidate the reason for inhomogeneous cell dispersal on certain PHOUs.

**REFERENCES:** <sup>1</sup>B. Witholt and B. Kessler (1999) *Curr. Opin. Biotechnol.* **10**:279-285. <sup>2</sup>M. Zinn, B. Witholt and T. Egli (2001) *Adv. Drug Del. Rev.* **53**:5-21.

**ACKNOWLEDGEMENTS:** The authors thank P. Manser for assistance.