Soft polymeric surface coatings made from olefinic medium-chain-length poly(3-hydroxyalkanoate)

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INTRODUCTION: *Pseudomonas putida* GPo1 is able to intracellularly accumulate medium-chain-length poly(3-hydroxyalkanoates) (mcl-PHAs) that consist of enantiomerically pure [R]-3-hydroxyfatty acids of between C6 and C14 carbon units. The polymer can be extracted, purified, and used as biodegradable and biocompatible polymer for medical applications[1]. Particular production conditions enable the tailored biosynthesis of functionalized mcl-PHA with terminal double bonds in the side-chains[2]. In this study we assessed the chemo-physical properties of mcl-PHAs produced from different mixtures of octanoic and 10-undecenoic acid and also investigated *in vitro* the adhesion of normal human dermal fibroblasts (NHDF) to polymer coatings.

METHODS: Poly(3-hydroxyoctanoate-*co*-3-hydroxy-10-undecenoate) was produced in a chemostat culture of *P. putida* GPo1 with tailored functionality (0, 10, 25, 50 and 100 mol% of terminal double bonds in the side-chains). The polymer was extracted, purified, characterized (GC, GPC and DSC), and solvent casted using methylene chloride on Petri dishes yielding coatings of at least 500 µm of thickness. For all cell experiments, the coatings were sterilized by heat at 80°C for 1 h, afterwards the coatings were stored at 4°C for 7 days for polymerization. Before cell seeding the coatings were incubated with 1 mL of cell culture media for 5.5 h (37°C, 95% air, 5% CO_2), the supernatant was then used for the cytotoxicity assay. NHDF (11.4 cells mm^{-2}) were applied onto the coatings) and incubated for 5 days (37°C, 95% air, 5% CO_2). On day 5 NHDF were stained for actin, vinculin and nucleus. The cytotoxicity assay was performed according to ISO-norm 10993-5: biological evaluation of medical devices, using the 3T3 mouse fibroblast cell line (ECACC No 85022108).

RESULTS: The molecular weight (Mw) of all PHAs were in the same range of 210 – 260 kDa but the melting properties differed significantly (Table 1). All polymers were hydrophobic and had a water contact angle above 85°. Interestingly, there were significant differences in the coverage of the cells on the coatings (Table 1). Best performing coatings were PHOUE (75/25; Fig. 1) and PHOUE (50/50).

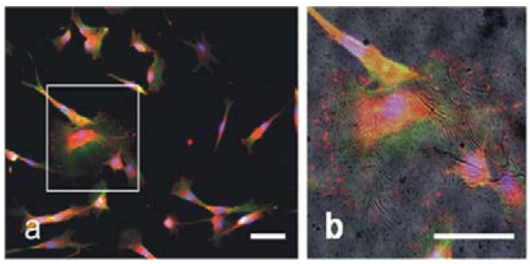

Fig. 1: NHDF stained for actin (green), vinculin (red), and nucleus (DAPI, blue) on PHOUE (75/25). a) fluorescence picture, b) overlay of fluorescene and light micrographs. Scale bar = 100 µm.

Table 1. Melting(Tm)glass transition (Tg) and adhesion of normal human dermal fibroblasts on the coatings after 5 days of incubation.

Polymer	Tm^2 [°C]	Tg^2 [°C]	NHDF P10 [cells mm^{-2}]
PHO (100)	58.1	-33.1	8.26 ± 3.19
PHOUE (90/10)	50.8	-35.9	9.24 ± 1.00
PHOUE (75/25)	44.5	-39.5	51.95 ± 5.41
PHOUE (50/50)	39.9	-44.6	42.58 ± 8.22
PHUE (100)	-	-49.3	12.29 ± 3.54

DISCUSSION & CONCLUSIONS: Despite the fact that all polymers were biocompatible, there were differences in the adherence to the coatings by NHDF. Light micrographs revealed that ruffle-like structures were formed surrounding the cells indicating that cells could exert forces to the substrates. Further studies are under evaluation in order to assess the reason for this particular effect.

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