CELL ADHESION ON SPIN-COATED POLY(BUTYLENE SUCCINATE-CO-DILINOLENE SUCCINATE) (PBS-DLS) COPOLYESTERS

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Introduction

Tissue engineering utilizes polymeric scaffolds to aid in the regeneration or replacement of damaged tissues or organs. In this context, we aimed observe and quantify the interactions between cells and new elastomeric poly(butylene succinate-co-dilinolene succinate) (PBS-DLS) copolyesters, in the form of thin, spin coated films on transparent substrates.

Materials and Methods

The obtained PBS-DLS copolymers were characterized using proton nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC), and gel permeation chromatography (GPC). Thin films were prepared from PBS-DLS_CAL-B synthesized with the use of enzyme *C.antarctica* lipase B (CAL-B) [3] and PBS-DLS_C-94 synthesized with the use of titanium oxide/silicon dioxide (C-94) [2], as well as commercial PCL (Sigma, M_n~80kDa) via spin coating on glass microscopy #1.5 coverslips. Samples were examined by Keyence VK9710 laser scanning (LSM) microscope (thickness, roughness) and Krüss drop shape analyzer. Cell adhesion experiments (3 h) were carried out with the use of L929 mouse fibroblasts. Cells were fixed, stained with Hoechst 33342, and imaged using Leica DMi8 inverted fluorescence microscope. Quantification was carried out using Fiji script, available on GitHub (psobolewskiPhD/ImageJ_Macros).

Results and Discussion

Appropriate choice of solvent permitted us to obtain uniform, repoducible thin films ~100 nm thick.

TABLE 1. Characterization of copolymers. Mn: number average molecular weight, Mw: weight-average molecular weight, $L_{BD\text{-}DLS}$: average sequence length, $X_{c,tot}$: total crystalline phase content, R: degree of randomness.

Copolymer	M_w [gmol^{-1}]	M_n [g/mol]	$L_{BD\text{-}DS.}$	$X_{c,tot}$ [%]	R
PBS-DLS 50:50_C-94	50 500	17 400	2.04	35.3	0.83
PBS-DLS 70:30_C-94	171 300	53 500	3.01	40.2	0.87
PBS-DLS 50:50_CAL-B	65 700	18 600	5.07	36.8	0.47
PBS-DLS 70:30_CAL-B	205 600	33 600	6.88	44.9	0.66

TABLE 2. Characteristics of films (n=2-4). Rq: root-mean-square.

Material	Thickness [nm]	Rq_{avg} [µm]	Water Contact Angle (°)	Spherulites [µm]
PCL	95	15	79	6.0
PBS:DLS 50:50_C-94	87	13	86	None
PBS:DLS 70:30_C-94	160	28	87	None
PBS-DLS 50:50_CALB	86	15	89	2.0
PBS-DLS 70:30_CALB	134	25	87	10.0

A clear difference in film morphology was observed between CALB and C-94 materials, with only the former exhibiting spherulites (likewise PCL).

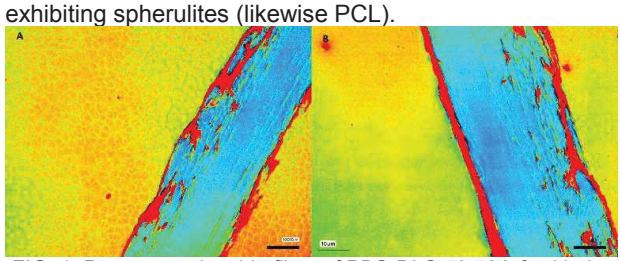

FIG. 1. Representative thin films of PBS-DLS 50:50 left with the use of CAL-B, right with the use of C-94 on coverslips. Scratches were used to measure film thickness.

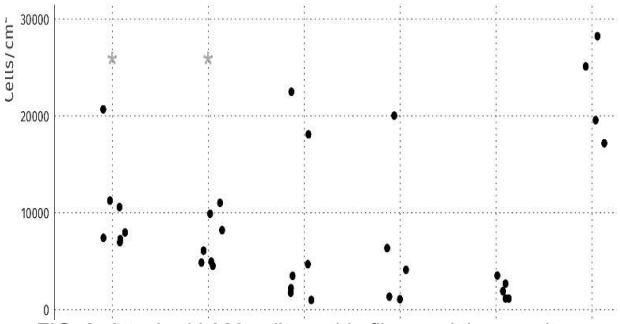

FIG. 2. Attached L929 cells on thin films and tissue culture plastic (TCPS) control after 3 hours. Stars indicate significant difference vs. PCL.

Statistical significance was tested in R software, using Kruskal-Wallis test, followed by Conover's non-parametric many-to-one comparison (two-sided, with Holm p-value adjustment) versus PCL. Post-hoc testing indicates that both PBSDLS5050 materials differed from PCL, which is in good agreement with prior provisional matrix adsorption study [1].

Conclusions

1. Choice of synthesis, enzymatic vs. polycondensation with TiO_2/SiO_2 (C-94) catalyst, influences copolymer block architecture.
2. Enzymatically synthesized copolymers have longer PBS sequences ($L_{BD\text{-}DS}$) than those synthesized using C-94 catalyst which facilitates spherulite formation.
3. Differences in polymer architecture (R, $L_{BD\text{-}DS}$) may explain observed differences in morphology of thin films.
4. Cell adhesion on PBS-DLS with the content of 50 wt% of soft segments was significantly higher than PCL, but no difference was observed between catalysts.

Acknowledgments

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