MC3T3-E1 Cell Responses to PCL/PEG Interfaces with Air or Different Substrates

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Statement of Purpose: Cell-biomaterial interactions are crucial for the applications of biomaterials. There are major categories of surface properties to determine these interactions.[1,2] In this study, we focused on both the interface with air and the one with the underlying substrate (glass, silicon, or Teflon) of isothermally crystallized poly(ε-caprolactone) (PCL) and its binary blends with 5% or 10% polyethylene glycol (PEG) (Fig. 1a). These interfaces had differences in roughness, ingredient components, and possibly chain orientation. We present surface roughness (R_q), water contact angle, and spherulitic morphologies of these spherulitic films and then discuss mouse pre-osteoblastic MC3T3-E1 cell attachment and proliferation on the spherulitic polymer films to demonstrate how the crystallization-induced interfaces on different substrates could affect cellular behavior.

Methods: Solutions of pure PCL and PCL/PEG blends with ϕ_{PEG} of 5% and 10% were prepared by dissolving 1 g of polymer in 10 mL of CH_2Cl_2. About 200 μL of the polymer solution was drop-coated onto glass coverslips, silicon wafers, and Teflon thin plates. After the polymer films on the substrates were fully dried in a vacuum oven, they were melted at 85 °C for 5 min and quickly transferred onto a hot stage for isothermal crystallization at 45 °C. Finally, the polymer films were peeled off and MC3T3-E1 cells were cultured for 4 days on the top surface, i.e., the interfaces with air and on the bottom surface, i.e., the one with the substrate and characterized.

Results: The spherulitic surfaces of PCL and PCL/PEG blends had different morphologies, hydrophobicities, and roughnesses when they crystallized on different substrates, as shown in Fig. 1b and Table 1. Good polymer spherulites were observed when the polymers contacted the glass and silicon substrates, while on Teflon, no obvious spherulites could be seen. The bottom interfaces of PCL and PCL/PEG blends on glass and silicon usually had lower roughnesses. The polymer films prepared on Teflon were much rougher than those on the other two substrates. The Teflon plate used here was also semi-crystalline and could affect the crystallization processes of PCL and PCL/PEG blends, probably leading to chain orientation.[3] Both MC3T3-E1 cell attachment and proliferation were better on the top surface of the polymer film than on the bottom surface when glass or silicon was used as the substrate. In contrast, no significant difference was found on the two surfaces when Teflon was used. The addition of PEG also improved both cell attachment and proliferation when glass or silicon was used. MC3T3-E1 cell attachment and proliferation were also significantly better on the top surfaces of the polymer films (and also the bottom surface for PCL) on glass/silicon than those values on Teflon.

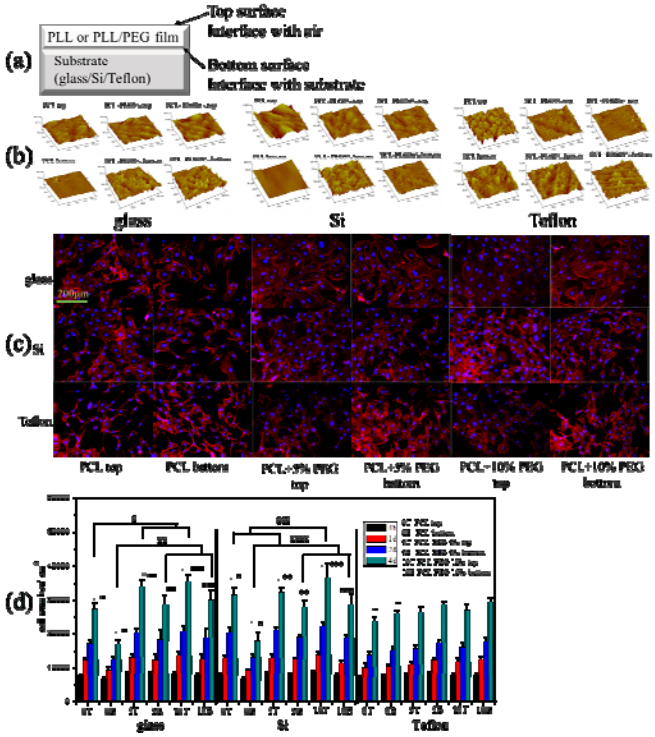

Figure 1. (a) Scheme of the polymer films coated on different substrates. (b) AFM images of the interfaces in the polymer films. (c) MC3T3-E1 cells on different polymer surfaces at day 2 post-seeding stained using rhodamine -phalloidin (red) and 4',6- diamidino-2-phenylindole (blue). (d) MC3T3-E1 cell densities at 4 h, days 1, 2, and 4. $^{+}$, *, $^{\$}$, $^{\#}$: $p < 0.05$ between any two samples marked with the same symbol. ^: $p < 0.05$ relative to the Teflon counterparts.

Conclusions: PCL and PCL/PEG spherulites with different interfaces on different substrates have been studied. Teflon has an effect on the crystallization of PCL and PCL/PEG. Distinct MC3T3-E1 cell attachment and proliferation were found on different interfaces both on glass and silicon, while there were no significant differences on Teflon. The cell attached and proliferated better on the top surfaces (and also the bottom surface for PCL) on glass/silicon than on Teflon.

References: 1. Wong J., *et al. Surf. Sci.* **2004**, 570, 119.
2. Saltzman W., *et al. In Principles of Tissue Engineering, 3rd Ed.* **2007**, 279-296.
3. Wittman J., *et al. Nature* **1991**, 352, 414.

Table 1. Surface roughnesses and water contact angles of the polymer samples

		Glass		Si		Teflon	
	sample	R_q (nm)	Contact angle(º)	R_q (nm)	Contact angle(º)	R_q (nm)	Contact angle(º)
top	PCL	54.4±8.5	74±1.4	53.4±3.4	76±0.6	132±21	74±1.7
	PCL+PEG5%	61.4±7.3	70±0.7	54.2±3.1	72±1.2	152±24	70±1.2
	PCL+PEG10%	64.3±5.5	69±1.0	52.4±3.7	69±1.3	128±14	67±1.5
bottom	PCL	18.2±4.0	70±0.7	6.0±1.4	71±1.3	157±10	72±0.6
	PCL+PEG5%	45.4±5.0	65±1.1	36.1±2.0	64±1.7	160±24	68±0.7
	PCL+PEG10%	43.6±3.5	63±0.9	37.1±2.3	64±0.9	174±27	68±1.1