Fibroblast Response to PLLA Nanotopographies is Enhanced by Edge Density and Proximity

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Statement of Purpose: Tissue engineering scaffolds require appropriate macroscale properties, e.g. mechanical characteristics, porosity etc. Scaffold micro- and nano-scale properties are increasingly recognized as important criteria influencing cellular response. Topography is known to affect the response but the mechanisms remain unclear. The correlation between fibroblast response and nanotopography geometry is investigated in this study.

Methods: A two-stage replication molding technique was used to texture poly(L-lactic acid) (PLLA) with nanotopographies created in heterogeneous polymer mixtures. Polystyrene (PS) and poly(4-bromostyrene) (PBrS) were blended 40%:60% ratio at concentrations of 5, 2 and 0.5% w/v in toluene. Aliquots were spin cast on glass substrates, inducing polymer demixing and surface topography of PBrS islands within a sea of PS. Smooth controls were created from 5% PS in toluene. Silicone elastomer was cast over these master topographies to form molds. Replicas were prepared by casting 2% PLLA in chloroform on to the molds. Surface topography was characterized by atomic force microscopy (AFM).

Human fibroblasts were seeded on to PLLA substrates. After 20min, 3hr, 1day, 3days and 7days samples were rinsed and fixed for subsequent analyses: staining to assess cell density and confluence; immunofluorescent labeling to assess actin microfilaments and focal contacts; dehydration for examining cell interaction with topographies via scanning electron microscopy (SEM).

Results/Discussion: PLLA topography was assessed via AFM (Fig1 and Table1). Topography size and proximity decreased with PS/PBrS concentration and topography edge density increased. Topographies from 5% PS/PBrS showed a bimodal distribution of large and small pillars.

Initial fibroblast response (20min, 3hr) was enhanced by nanotopographies, especially on 2 and 0.5% substrates (Fig 2). At 3hr, focal complex formation was observed at cell peripheries on textured substrates, colocalized with actin microfilament formation. At later times cell density and confluence were decreased on 5% substrates. Cytoskeletal development was poorest on 5% substrates with limited focal adhesions and indistinct microfilaments observed. Cells on smooth PLLA had large, well-formed adhesions, while those on 2 and 0.5% substrates tended to be smaller but more numerous. Cytoskeletal development correlated with cell response, with cell density increased on substrates supporting focal adhesion development.

SEM revealed cells on 2% and 0.5% substrates with pseudopods on and between islands. Pseudopods on 5% substrates were typically limited to inter-island regions.

Cell response trended with topography proximity and edge density. It is hypothesized that 2% and 0.5% topographies, while smaller than typical focal adhesions, allow sufficient integrin clustering for focal adhesion formation, with adhesions spanning topographies or forming across topographies and the intervening substrate. It is possible that the trend with edge density may be due to protein conformational changes occurring at surface discontinuities, as hypothesized by others.

Conclusions: This study provides further evidence that surface topography may affect cellular response and suggests geometric properties that may govern this effect. It is anticipated that further work with controlled variation of geometry will provide additional insight.

Acknowledgements: This work was funded by Dept of Surgery Feasibility Grants and the Dorothy Foehr Huck and J. Lloyd Huck Institutes of Life Sciences. Assistance was provided by Dept of Orthopedics and Rehabilitation.

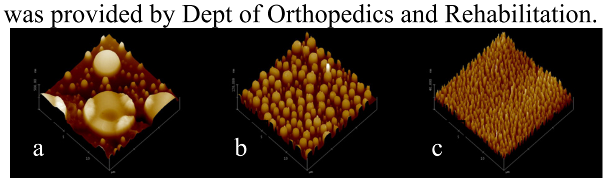

Figure 1. 15 x 15 μm AFM images of PLLA samples replicated from a) 5%, b) 2%, c) 0.5% PS/PBrS

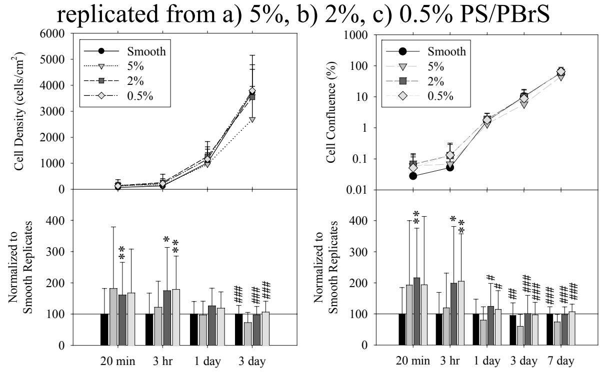

Figure 2. Variation in cell density and confluence with time (significance to * smooth and # 5% PLLA)

Table 1: Geometric properties of PLLA topographies replicated from 5, 2 and 0.5% PS/PBrS demixed polymers (mean±SD)

	R_{rms} (nm)	Δ Sample Area (%)	Height (nm)	Topography Area (μm^2)	Effective Radius (nm)	Center-Center Spacing[a] (nm)	Inter-Topography Spacing[a] (nm)	Edge Density (μm^{-1})
5 %[b]	52.2 ± 4.3	1.10 ± 0.15						0.59 ± 0.16
≥ 3 μm^2			125.9 ± 12.7	12.8 ± 11.3	1900 ± 700	2850 ± 750	500 ± 400	
< 3 μm^2			83.7 ± 14.1	0.59 ± 0.37	400 ± 100	1750 ± 850	750 ± 750	
2 %	11.3 ± 0.5	0.49 ± 0.03	30.9 ± 2.4	0.52 ± 0.26	400 ± 100	900 ± 175	125 ± 150	2.63 ± 0.15
0.5 %	3.5 ± 0.3	0.18 ± 0.06	10.7 ± 1.4	0.12 ± 0.07	180 ± 60	470 ± 130	100 ± 90	4.77 ± 1.13

[a] spacing assessed for each feature and its 3 nearest neighbors [b] 5% samples presented as gross, large and small area features