Nanoscale Poly(L-Lactic Acid) Surface Texture Influences Fibroblast Response

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Introduction: The application of poly(α-hydroxy esters), such as poly(L-lactic acid) (PLLA), to tissue engineering scaffolds is well known. Cell response to biomaterials is key, and both ordered and random surface textures have been shown to affect this. Ordered textures (i.e. having symmetry) are most often microfabricated in materials having little biomedical applicability (e.g. silicon and quartz). This study presents a technique for replicating textures in PLLA. Ordered and random, sub-micron and nanoscale textures are characterized and the response of fibroblasts is assessed and compared to smooth controls.

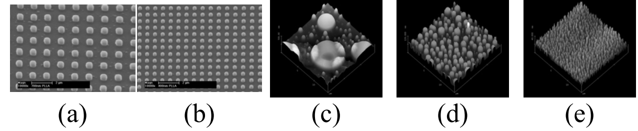
Methods: PLLA was textured via a soft lithographic two-stage replication molding technique[1]. Briefly, a master pattern is created, a silicone mold is cast and PLLA is cast in to the mold to replicate the master. Both ordered and randomly textured masters were created. Ordered master topographies were prepared by Stepper lithography microfabrication: pillars with 700nm width and spacing (pillar area ~0.4μm^2), and with 400nm width and spacing (~0.1μm^2), with heights of ~650nm. Random master topographies were prepared by polymer demixing: spin casting 5, 2 and 0.5% w/v in toluene of a 40% polystyrene (PS) and 60% polybromostyrene (PBrS) blend, resulting in randomly distributed PBrS pillars on PS. Human foreskin fibroblasts were seeded in Minimum essential medium (Eagle) with 10% fetal bovine serum and incubated at 37ºC in 5% CO_2. After 20min, 3hr, 1day, 3days and 7days, samples were rinsed, fixed, stained and cell numbers and confluence were assessed via optical microscope. Focal contacts/adhesions (FA) and actin microfilaments (MF) were labeled with anti-vinculin and phalloidin respectively.

Results / Discussion: Ordered PLLA pillars were replicated with yield of nearly 100% (Fig 1a-b). PLLA replicas of random demixed polymers had topographies with distributions of heights and diameters: 5% blends had mean height of 120nm and diameter of 1.45μm, 2% were 30nm by 740nm, and 0.5% were 9nm by 380nm (Fig 1c-e). On ordered pillars, cell adhesion (up to 1day) was higher than on smooth PLLA. This was statistically significant at all times (20m, 3hr, 1d) for 400nm pillars, but only at 3hr for 700nm pillars (Fig 2). Proliferation was reduced on pillars, with significantly higher cell density and confluence on smooth PLLA at 3 and 7d. Increased FA labeling was observed at pseudopodia edges on pillars suggesting focal complex formation (Fig 3a), with MF formation occurring in these areas. At higher time points fibroblasts on smooth PLLA exhibit large, well-defined FA (Fig 3b) localized at the ends of distinct MF. Pillars limited FA to inter-pillar regions, with FA frequently aligned in the pillar direction (Fig 3c), and MF development was poorer. Cells were sometimes found to deform 400nm pillars (Fig 3d). It appears that focal complexes (area <1μm^2) may form on the pillars allowing and possibly increasing initial adhesion perhaps via increased surface area. However, mature FA (area >1μm^2) cannot form on the pillars, affecting links between the actin cytoskeleton and extracellular matrix. This is consistent with the increased adhesion but reduced proliferation observed on the ordered pillars. Preliminary results assessing response to random textures of less height than the pillars show similar trends with increased initial adhesion (Fig 4). Proliferation is reduced on the 95nm high PLLA replicas but confluence on 30nm and 9nm textures is comparable to smooth.

Conclusions: The ability to influence cell adhesion and proliferation is key to tissue engineering. This study shows that surface texture may increase initial adhesion, possibly via increased focal complex formation, and texture height appears to play a role in later proliferation by restricting the formation of focal adhesions.

References:

1. Milner KR. ASAIO J. 2005;51:578-584; J. Biomed. Mater. Res. A (in press)



(a) (b) (c) (d) (e)

Fig 1: SEM Images of (a) 700nm and (b) 400nm PLLA pillars and 15 μm^2 AFM images of (c-e) PLLA replicas of 5, 2 and 0.5 % PS/PBrS masters

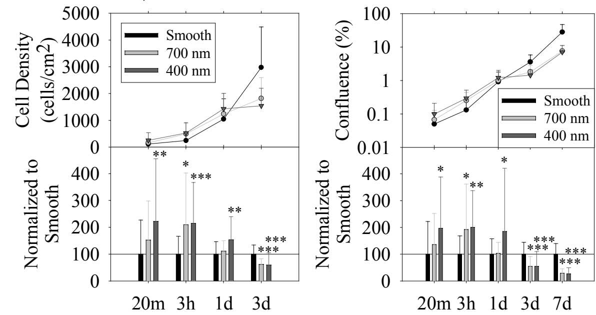


Fig 2: Variation in cell density and confluence with time

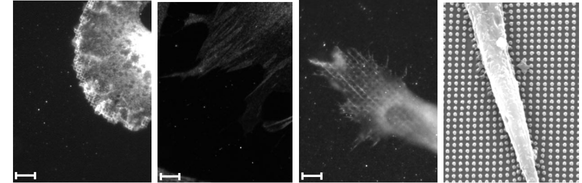


Fig 3: Immunofluorescent images of FA on 400nm PLLA at (a) 3hr and (c) 7d and on smooth PLLA at (b) 7d (5μm scale). (d) SEM of fibroblast on 400nm PLLA at 7d

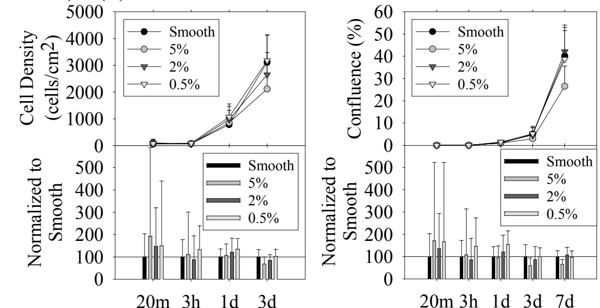


Fig 4: Variation in cell density and confluence with time