Combinatorial Investigation of Osteoblast Response to Nano- and Microstructured Biomaterials

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Introduction: One ultimate goal of biomaterial surface research is to understand how the surface chemistry and structure of the material can be used to control cell response. However, surface chemical and physical properties, the essentially infinite number of surface patterns, plus the stochastic nature of cell responses challenges traditional one-sample-one-measurement methods. Combinatorial libraries of biomaterials (both discrete and gradient) and high-throughput techniques have been developed and employed recently for efficiently screening biomaterials for cell attachment, proliferation and differentiation. These methods allow 100 to 1000 chemistries and surface patterns to be quantitatively studied on a single "chip". We report here on separate studies of osteoblast attachment and proliferation on combinatorial libraries of polyester blends, polyurethane-polyester blends, and semi-conducting polymers [3].

Materials and Methods. Solutions of poly(DL-lactide-co-glycolide) (PLGA, Birmingham Polymers), poly(e-caprolactone) (PCL, Aldrich), poly(ethylene glycol) (PEG), and poly(3octylthiophene-2,5-diyl) (P3OT) were prepared in chloroform. A mixture of 4,4 methylene bis-phenyl diisocyanate (MDI, Sigma-Aldrich) and Pluracol® (BASF) were prepared in tetrahydrofuran (THF, EMD). MC3T3-E1 Subclone 4 pre-osteoblastic cells (MTCC) and MC3T3-E1 cells (Riken Cell Bank) were used.

Library Preparation. The ***PLGA/PCL*** libraries (22mm × 22mm) were prepared by coating HF processed silicon wafers with PLGA-PCL composition gradient films (from 0 to 70 mass %), followed by heating along a temperature-gradient from 130 to 90 ºC for 2 hours using well-established procedures described in detail elsewhere[1]. The ***PEG-MDI-Pluracol®/PCL*** libraries were prepared in similar methods while the annealing was in the temperature gradient from 80 to 120 ºC for overnight. ***P3OT*** constant thickness (22mm×22mm, thickness ~150nm) and gradient thickness (32mm×32mm, thickness: 120 to 200 nm) libraries were prepared on silicon wafers as described in [4].

In situ culture and assay: MC3T3-E1 cells were cultured on libraries using standard procedures. Following cell culture and fixation at various time-points, the libraries are stained for counting cell density (Hoechst 33342), proliferation (immunohistochemically stained BrdU) and cell shape (F-Actins as markers, stained with Phalloidin-TRITC). Crossed-polarized optical microscopic images were taken of each region together with fluorescent ones. Usually an equally-spaced grid of 16 to 360 images was collected on each library. Images were processed and analyzed with the Image Processing Toolbox of MatLab™ 7.0 and ImageJ.

Results and Discussion. Round PCL islands are the dominating patterns on PLGA/PCL libraries. Individual cell based metrics, naturally connected to Bayesian analysis, was established for investigating cell-PCL interactions locally. For each cell in each image, cell-to-PCL distance and the relative PCL island size is measured for both proliferating cells and all cells, and the summary distributions were normalized into frequency functions $f_{PC}(d_{Cell-to-PCL}, dia_{PCL})$ (for proliferated cells) and $f_{AC}(d_{Cell-to-PCL}, dia_{PCL})$ (for all cells). The frequency functions quantitatively described the local environment of cells. The difference of f_{PC} and f_{AC} was used for probing the specific PCL features that promotes cell proliferation.

The localized analysis (Figure 1) showed that specific combinations of PCL island size and cell-to-PCL distance promote cell proliferation. Based on this observation, a shaper/holder model is proposed. Cell proliferation is apparently influenced by a specific combination of large islands that constrain cell spreading (shapers) and small islands that provide cell attachment sites (holders). Specific combinations of shapers and holders can significantly improve of suppress cell proliferation.

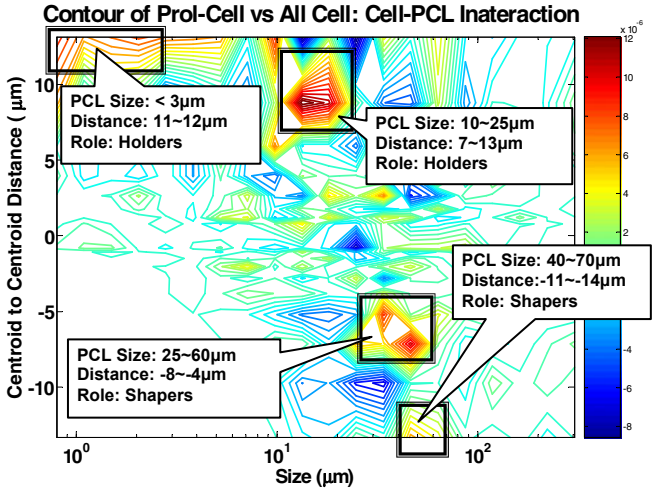

Figure 1. Localized Analysis of Cell Proliferation of PLGA/PCL Libraries

On PEG-MDI-Pluracol / PCL libraries novel PCL phases with ring-like morphology (Figure 2-insert) were discovered. Effects of PCL island size on cell attachment were analyzed and smaller PCL islands (Figure 2) were associated with increased cell attachment.

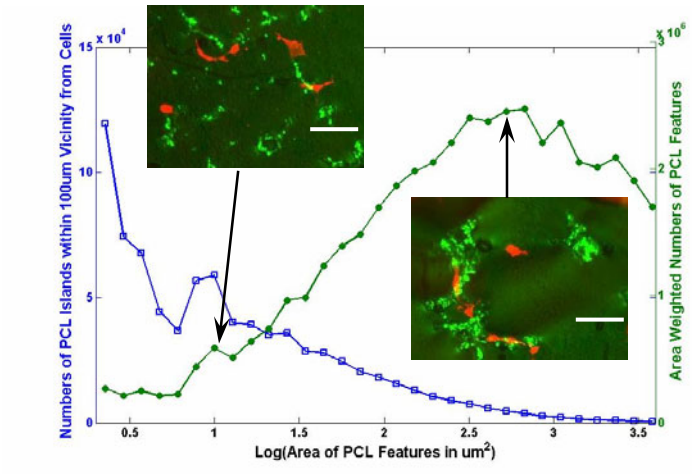

Figure 2. PCL feature size distribution (green solid circles) and cell attachment distribution on PEG-MDI-Pluracol/PCL Library (blue open squares). *Inserts:* Green=PCL phase; red= cell actin, bar = 100μm)

Conclusions. Combinatorial biomaterial screening is a powerful tool for exploring surface effects on cell behaviors. A shaper/holder model is proposed from PLGA/PCL libraries. Novel ring-like or island-like phase separation patterns were discovered from PEG-DMI-Plurocal/PCL libraries. Cells were found to attach more near small PCL features than larger ones. Preferential attachment based on shape, instead of size, will be studied further.

References

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