Gradient Libraries for Combinatorial and High-Throughput Investigations of Polymeric Biomaterials

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Introduction

On the scale of complexity, synthetic or processed polymeric materials are rather simple. Nevertheless, assessing the range of complex interplay that characterizes the interactions of cells with synthetic materials presents a challenge for tissue engineering. The gradient library approach to combinatorial and high-throughput analysis of these interactions provides a powerful tool. Just as the combinatorial methodology has changed the paradigm of pharmaceutical assessment, and it is moving to have a similar impact on traditional materials science, we are working at the interface. Unlike the combinatorial synthetic approach, the NIST combinatorial approach emphasizes high-throughput methods to generate experimental data over the multi-parameter space.

In the complexity of biomaterials and their applications our efforts have evolved into three interwoven tool sets to explore cellular response to materials: diverse material library preparation, high-throughput screening and assays, and statistical analysis of discrete population distributions. This talk will focus on the application of high-throughput techniques used to screen polymeric materials with gradients libraries.

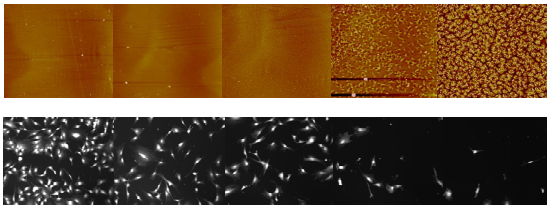
Experimental

Methods have been developed that allow preparation of gradients of polymer blend composition, blend phase morphology, crystallinity, copolymer microstructure, surface energy, pattern texture and chemical composition, which could then be characterized by high-throughput or traditional materials analysis.[1] For example, we have prepared T-ϕ combinatorial libraries consisting of blends of poly(D,L-lactide) and poly(ε-caprolactone). These blends are ideal for the combinatorial assay of the effects of T and f on cell adhesion and proliferation, because these polymers exhibit T-ϕ dependent lower critical solution temperature phase behaviour.[2] The temperature quenched phase libraries were analysed by high-throughput screening with FTIR spectroscopy, and optical, and atomic force microscopy (AFM) for rapid and efficient characterization of polymer blend composition, microstructure and phase behaviour . UMR-106 osteosarcoma cells were cultured on the T-ϕ libraries in order to assay cell adhesion and proliferation, and correlate cell behaviour to polymer microstructure, crystallinity, composition, and phase behaviour.

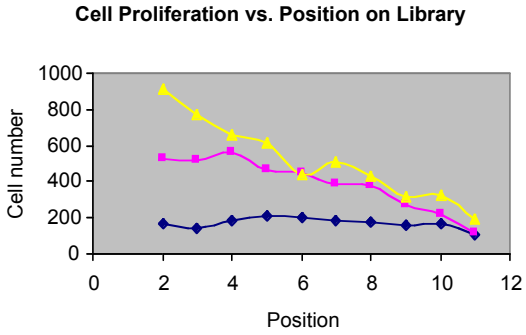
In a similar manner, poly(L-lactic acid) was carefully purified and film coated on a polished silicon wafer. Film thicknesses ranged from 250 nm to 350 nm as measured using a automated interferometer. Samples cut into 6 cm x 1.5 cm coupons and annealed to allow controlled crystallization of the film on a temperature gradient stage with the limits of the stage held at 44 ºC and 100 ºC. The linearity of the temperature gradient was verified using a thermocouple and the homogeneity of the resultant crystallinity gradient was verified using optical microscopy. For the cell proliferation studies MC3T3-E1 cells were added to wells containing the gradient libraries and given time to settle and to form preliminary attachments before transporting the flask to the incubator. Samples were cultured for 1, 3, or 5 d.

Results

In all cases the first level of analysis is to assay cell proliferation. It is shown that the rate of proliferation on the smooth regions of the films is much greater than that on the rough regions and a monotonic variation in rate is observed as a function of roughness. The critical roughness for which a statistically significant reduction in rate of proliferation occurs was 4 ± 1 nm. Results from ELISA experiments indicate no significant change in the conformation or concentration of adherent proteins, suggesting the cells were directly responding to substrate topography. Representative surface characterization data and fluorescent microscopy are shown below.[3]



Above: Montage of representative images of PLLA morphology from AFM data (top panel) and corresponding cell count from fluorescent microscopy (bottom panels). Below: Representative plot of cell count as function of position across gradient library.



References

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2. J.C. Meredith, J.L. Sormana, B.G. Keselowsky, A. Garcia, A. Tona, A. Karim, and E.J. Amis, "Combinatorial Characterization of Cell Interactions with Polymer Surfaces", *J. Biomed. Mater. Res.,* 2003, **66A,** 483-490.

3. N.R. Washburn, K.M. Yamada, C.G. Simon, S.B. Kennedy, and E.J. Amis, "High-throughput Investigation of Osteoblast Response to Crystalline Polymers: Influence of Nanometer-scale Roughness on Proliferation", *Biomaterials, in press.*