Combinatorial Screening of Cell-Material Interactions

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INTRODUCTION

Tissue engineering applications involve developing materials to guide cellular response in the repair of missing or damaged tissue. We have been developing combinatorial methods for characterizing the functional dependence of cellular responses on material properties. Two strategies were pursued: gradient libraries, where samples are prepared with systematic variations in material properties, and combinatorial libraries, where concepts of full- and partial-factorial design are used to screen the interplay between biochemical, material, and cellular variables. A brief overview of our work in these areas will be presented. Cellular responses to gradient libraries of polymer crystallinity, blend composition, and surface energy have been investigated. We have characterized proliferation and matrix production of MC3T3-E1 osteoblasts as a function of material variables and are developing methods for measuring these responses using fluorescence microscopy. Design-of-experiment methods have been used to characterize the interactions of chondrocytes with peptide-functionalized alginates. Using these methods we have also characterized the influence of growth factors known to be important in cartilage development in vitro. The influences of insulin-like growth factor-I (IGF-I), transforming growth factor- β (TGF-β), and basic fibroblast growth factor (bFGF) were investigated as a function of alginate functionalization and cell density.

EXPERIMENTAL

Reagents. Poly(L-lactic acid) (PLLA) and poly(D,L-lactic acid) were purchased from Polysciences, separately dissolved in chloroform (Sigma) at concentrations of 2 % (g/g), and passed through a 0.2 μm filter. Chlorodimethyloctylsilane was purchased from Aldrich and used as received. A 1.2 % sodium alginate solution (mass fraction) was prepared by dissolving 1.2 g of Pronova UP LVG alginate (64 % gluronic acid; Pronova Biomedical; Oslo, Norway) in 100 mL of saline (155 mmol/L NaCl).

Preparation of gradient libraries. Gradient libraries of crystallinity were prepared by flow-coating 250 nm films of PLLA onto polished silicon wafers (WaferWorld), drying for 14 d, then annealing on a temperature gradient with the stage ends held at 44 ºC and 100 ºC for 3 min. Composition gradients of PLLA and PDLLA were prepared by depositing a stripe of polymer solution having a gradient in relative composition and flow-coating across a polished silicon wafer. MC3T3-E1 osteoblasts (ATCC) were seeded on the scaffolds at a density of 50,000 cells/mL in Dulbecco's Modified Eagle Medium (Gibco) supplemented with 5 % fetal bovine serum (Gibco).

Characterization of gradient libraries. Gradient library topography was investigated with an atomic force microscope (Digital Instruments) operating in tapping mode. Polymer morphology was measured by averaging at least three (50 μm x 50 μm) regions of the library with identical composition and processing conditions. Polymer surface energy was characterized using water contact angle measurements. Composition of the gradient library was measured by infrared microscopy.

Certain commercial materials and equipment are identified in this paper in order to adequately specify the experimental procedure. In no case does such identification imply recommendation by the National Institute of Standards and Technology nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

Preparation of combinatorial libraries. Solutions were mixed at the following concentrations in 24-well plates (Daigger):

Variable	High value	Low value
Cells per mL	30,000	3000
IGF (ng/mL)	100	1
TGF-b (ng/mL)	1	0.01
bFGF (ng/mL)	10	0.1

Calcium-free alginate solutions containing 5 % fetal bovine serum (v/v) were added to these solutions with 100 mM $CaCl_2$ to promote gelation.

Characterization of cellular proliferation. Proliferation on gradient libraries was measured by fixing cells in 4 % solutions (v/v) of formaldehyde (Aldrich) in Hank's balanced salt solution (Gibco). Cells were then stained with DAPI (Sigma) and counted in a spatially registered method using a fluorescence microscope with a computerized translation stage (Leica). Cell density as a function of position was calculated and correlated with the previous measurements on material characterization. Proliferation in the combinatorial libraries was measured by determining the DNA content of enzymatically-digested scaffolds using a PicoGreen Assay (Molecular Probes) and a fluorescence microplate reader (Wallac 1420 Victor2, PerkinElmer Life Sciences).

RESULTS AND DISCUSSION

The goal of these experiments was to determine the functional dependence of cellular responses on material parameters. As an example, in Figure 1 are shown a series of representative images of polymer topography and cell number after three days.

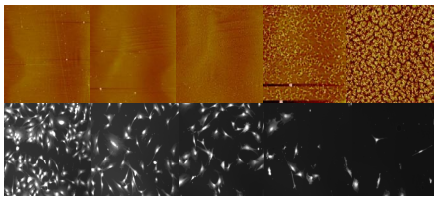

Figure 1. Representative AFM (top) and fluorescence microscopy (bottom) images depicting cell proliferation as a function of polymer crystallinity.

Cell proliferation decreases monotonically as a function of surface roughness, and MC3T3-E1 osteoblasts were found to be sensitive to rms roughness values of 1.1 nm (as compared to 0.8 nm for amorphous PLLA).

Chondrocyte proliferation in alginate hydrogels was characterized by calculating the interaction effects matrix as a function of growth factor concentration and cell density. The following results were obtained for unfunctionalized alginates:

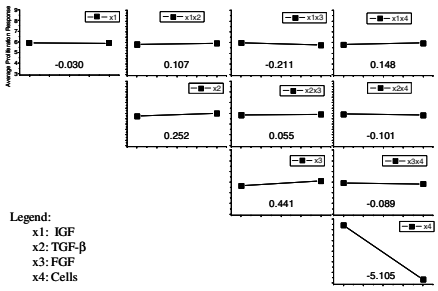

Figure 2. Interaction effects matrix for alginate-seeded chondrocytes.

The strongest variable appears to be cell density, and suggest proliferation at higher cell density is strongly inhibited. These data also suggest there are extensive couplings between growth factors that determine cellular proliferation.