

## SURFACE ENGINEERING OF MATERIALS FOR BIOMIMETIC IMPLANTS AND CELL-BASED SENSORS

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**INTRODUCTION:** A central theme in the design of materials that actively regulate the response of either mammalian cells or tissue formation is that they do so through a combination of biomolecular recognition processes and device micro-architecture. In my research group we have designed and synthesized model biomimetic materials that can be used to test hypotheses regarding cell-materials interactions. We can control cell behaviour chemically either through specific ligand-receptor interactions or by modifying the projected area of cells to limit growth and promote differentiation. These surfaces may find use in applications such as combinatorial analyses, novel culture systems, the modification of implant surfaces, DNA chips, and cell-based biosensors. In this work we have elicited these materials to begin to understand the details in the cascade of events involving cell morphology, cytoskeletal organization, intracellular signalling, nuclear shape, nuclear matrix organization, promoter geometry, and gene expression. We report the first method for both precisely controlling cell and nuclear shape, and measuring in situ mRNA expression and collagen I expression in an effort to help elaborate these mechanisms.

**METHODS:** We have developed methods that incorporate photolithography, organosilane chemistry, photoinitiated polymerisation, and peptide chemistry to create surfaces that control the spatial distribution, projected area, and nuclear shape of mammalian cells. For example, we synthesized novel interpenetrating polymer networks (IPNs) based on polyacrylamide [p(AAm)] and poly(ethylene glycol) [PEG] that form thin coatings (~ 20nm) on both metal oxides (e.g., SiO<sub>2</sub>, TiO<sub>2</sub>) and polymers (e.g., polystyrene). We have determined that these IPNs prevent protein adsorption and cell adhesion and therefore represent an excellent surface to control the spatial distribution of either biological macromolecules, cells, or viruses. Patterned surfaces were fabricated using a photolithographic process resulting in islands of cell binding N-(2-aminoethyl) - 3 - aminopropyl - trimethoxysilane (EDS) separated by a non-adhesive IPN

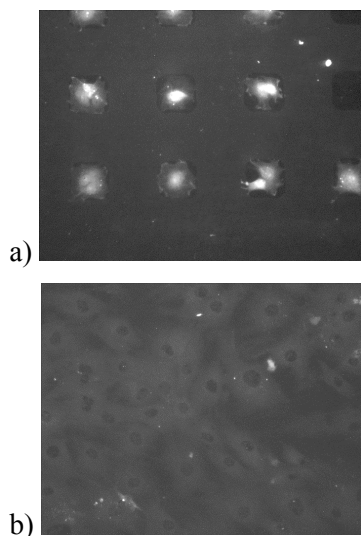
[poly(acrylamide-co-ethylene glycol); p(AAm-co-EG)] as described previously.<sup>1</sup> The surfaces contained over 3800 adhesive islands/cm<sup>2</sup>, allowing for isolation of single cells with projected areas ranging from 100 μm<sup>2</sup> to 10,000 μm<sup>2</sup>. Surfaces were characterized using ToF-SIMS with imaging capabilities. Additionally, protein adsorption from serum was analysed using immunostaining and confocal microscopy.

Bone cells were isolated from rat calvaria (6-12 days old) and exposed to the surfaces. Cytoskeletal organization was examined, using F-actin staining, to determine the influences of confining cell projected area on stress fibre formation. Determination of single cell protein expression on these surfaces required a reverse transcriptase in situ polymerase chain reaction (RT in situ PCR) be performed. Therefore, protein expression at the mRNA level could be observed within single cells with 56 different projected areas (>12,000 cells) on one surface. In particular, osteocalcin expression was examined within primary bone-derived cells exposed to these chemically patterned surfaces. Collagen I synthesis was determined intracellularly via indirect immunofluorescence microscopy.

**RESULTS:** ToF-SIMS with imaging capabilities verified that the photolithographic preparation of the surface resulted in spatially-resolved chemistries. Preferential adsorption of Vn to the EDS regions directed the distribution and projected area of mammalian cells.<sup>2</sup>

Cell attachment, distribution, and spreading was dictated by the EDS chemistry, resulting in single cell attachment to regions <2000 μm<sup>2</sup>. Larger EDS regions supported multiple cell attachment and/or cell proliferation. F-actin staining indicated that stress fibre formation was influenced by the shape of the adhesive EDS region. The shapes of the nuclei of primary osteogenic cells were also controlled on microfabricated substrata. Gene expression and protein synthesis were altered by simply changing the shape of nucleus, dictated by the island size, without the use of chemical intermediates or changing the surface density of adsorbed

extracellular matrix proteins. Collagen I synthesis correlated directly with cell shape and nuclear shape index (NSI), where intermediate values of nuclear distension ( $6 < \text{NSI} < 8$ ) promoted maximum synthesis. Osteocalcin mRNA, a bone-specific differentiation marker, was observed intracellularly using RT in situ PCR at 4 days in cells constrained by the pattern and not detected in unconstrained cells of similar projected area, but different NSI (Fig. 1). These data support the concept of “architectural” transcription factors that promote gene expression based on optimal stress within the nuclear matrix transduced by the cytoskeleton.



*Figure 1: RT in situ PCR for osteocalcin on a.) EDS/P(AAm-co-EG) and b.) homogeneous EDS surfaces. Light regions indicate presence of osteocalcin mRNA.*

**DISCUSSION & CONCLUSIONS:** Our data supports the concept of gene expression and protein synthesis based on optimal distortion of the nucleus, possibly altering transcription factor affinity for DNA, transport to the nucleus, or nuclear matrix organization. The combination of microfabricated surfaces, RT in situ PCR, and NSI measurement is an excellent system to study how transcription factors, the nuclear matrix, and the cytoskeleton interact to control gene expression and may be useful for studying a wide variety of other cell shape/gene expression relationships

**REFERENCES:** <sup>1</sup> Thomas, C.H., et al., JBME, 121:40, 1999. <sup>2</sup>Thomas, C.H., et al., JBMR, 37:81, 1997.

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