Technologies for Protein Analysis and Tissue Engineering, with Applications in Cancer

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A man should look for what is, and not for what he thinks should be.

-Albert Einstein

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Abstract

The first part of this thesis describes electrolyte transport through an array of 20 nm wide, 20 μm long SiO_2 nanofluidic transistors. At sufficiently low ionic strength, the Debye screening length exceeds the channel width, and ion transport is limited by the negatively charged channel surfaces. At source-drain biases > 5 V, the current exhibits a sharp, nonlinear increase, with a 20 − 50-fold conductance enhancement. This behavior is attributed to a breakdown of the zero-slip condition. Implications for peptide sequencing as well as energy conversion devices are discussed.

The next part describes a technology for the detection of the highly aggressive brain cancer glioblastoma multiforme (GBM). In general, proteomic approaches have shown great promise in recent years for correctly classifying and diagnosing cancer patients. However, no large antibody-based microarray studies have yet been conducted to evaluate and validate plasma molecular signatures for detection of glioblastoma and monitoring of its response to therapy. In this study, we compared plasma samples from 46 glioblastoma patients (72 total samples) with those of 47 healthy controls with respect to the plasma levels of 35 different proteins known to be generally associated with tumor growth, survival, invasion, migration, and immune regulation. Average-linkage hierarchical clustering of the patient data stratified the two groups effectively, permitting accurate assignment of test samples into either GBM or healthy control groups with a sensitivity and specificity as high as 90 % and 94 %, respectively (when test samples within unbiased clusters were removed). The accuracy of these assignments improved (sensitivity and specificity as high as 94 % and 96 %, respectively) when the cluster

analysis was repeated on increasingly trimmed sets of proteins that exhibited the most statistically significant ($p < 0.05$) differential expression. The diagnostic accuracy was also higher for test samples that fell into more homogeneous clusters. Intriguingly, test samples that fell within perfectly homogeneous clusters (all members belonging to the same group) could be diagnosed with 100 % accuracy. Using the same 35-protein panel, we then analyzed plasma samples from GBM patients who were treated with the chemotherapeutic drug Avastin (Bevacizumab) in an effort to stratify patients based on treatment-responsiveness. Specifically, we compared 52 samples from 25 patients who exhibited tumor recurrence with 51 samples from 21 patients who did not exhibit recurrence. Again, several proteins were highly differentially expressed and cluster analysis provided effective stratification of patients between these two groups (sensitivity and specificity of 90 % and 96 %, respectively).

Finally, single-cell resolution patterning of tissue engineered structures is demonstrated. The proper functioning of engineered constructs for tissue and organ transplantation requires positioning different cell types in anatomically precise arrangements that mimic their configurations in native tissues. Toward this end, microfabrication strategies have facilitated great strides in cell micropatterning in recent years, but these technologies are still limited in that they can typically only pattern one or two cell types at a time with feature sizes that are larger than a single cell. We present a patterning methodology that allows for high-density, multiplexed patterning of distinct cell types on glass at single-cell resolution. The technique involves two microfluidic-patterning steps run perpendicularly to each other in which “anchor” oligos are first laid down on a polylysine-coated glass substrate followed by binding of “bridging” oligos

containing both an anchor-complementary sequence and a unique sequence that can bind to an oligo-functionalized cell. In this manner, dense arrays of 3 x 3 and 3 x 1 DNA grids can be patterned and then converted into cell arrays. As a proof-of-concept, both a neuron-astrocyte construct and a pancreatic islet construct containing 2 distinct islet cell types were patterned separately as a dense array of cell grids. Once fixed in a hydrogel matrix, layers of patterned cells were then stacked to form 3-D tissue engineered constructs.

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