Investigating the Effect of ECM Proteins on Substrate-Mediated Virus Gene Delivery

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Statement of Purpose: Complex tissue development requires precise control over the spatial organization of gene expression. Tissue engineering strategies have expanded to overcome significant barriers, but creating spatially controlled gene expression profiles in an engineered construct is still difficult. We have developed a platform to allow for both viral gene delivery vectors and adhesive proteins to be co-patterned to control both cellular attachment and gene expression, which may ultimately enable us to modulate the virus pattern underneath the cell pattern, resulting in the creation of distinct regions of gene expression within the population. We have created this technology based on adeno-associated virus (AAV) to enable tissue engineers to obtain specific, spatially organized expression of genes in a population of cells. This gene delivery vector can be easily tailored to deliver genes into target cells with high efficiency. We are capable of localizing both cellular adhesion and gene expression using polydimethylsiloxane (PDMS) stamps to pattern alkanethiol solutions onto gold-coated substrates. We have observed a synergistic effect between the patterning of human fibronectin (HFN) and AAV for the delivery of genes to HeLa cells. Here, we further explore this technology by examining additional adhesive proteins. We show that collagen I, elastin, laminin, and poly-l-lysine are also compatible.

Methods: We patterned alkanethiol solutions with a variety of functional groups in order to create different surface chemistries, including $-CH_3$ (hydrophobic), -COOH (negatively charged), $-NH_2$ (positively charged), and -OH (hydrophilic) to determine the optimal surface conditions for our technology. Circular patterns with 500 micron diameters were printed onto gold-coated glass coverslips and then backfilled with an -OEG terminated alkanethiol to create a protein resistant background. Patterns were then exposed to either virus or ECM protein followed by virus. Samples were then either immunostained to analyze adsorption of both ECM protein and viral particles or seeded with HeLa cells at 100 cells/mm^2.

Results: We have demonstrated the ability to spatially localize both cellular adhesion protein (HFN) and viral gene delivery vectors. We have previously created islands of HFN and AAV2-GFP (recombinant virus delivering a cassette for GFP) with a 500 micron diameter. Using immunostaining, we find that AAV2 preferentially attaches to $-CH_3$ patterns with the highest efficiency. However, when HFN is adsorbed first, followed by AAV2, both $-CH_3$ and $-NH_2$ surfaces yield high levels of viral attachment. Co-adsorbed patterns were also seeded with HeLa cells, which are permissive to AAV2 infection. Cell adhesion and gene expression were monitored over three days and we observe that HFN and AAV2 co-patterns are capable of facilitating robust patterns with high gene expression on $-CH_3$, -COOH, $-NH_2$ surfaces. The -OH surfaces were deemed unsuccessful due to low cellular adhesion. Most interestingly, we discovered a synergistic relationship between HFN pre-adsorption and AAV2 gene delivery. Gene delivery is significantly higher when HFN is pre-adsorbed, in comparison to virus directly immobilized onto alkanethiol surfaces.

Ultimately, in order for our technology to be successful, our surfaces must be capable of both localizing cells and facilitating high gene delivery. To further develop our platform we have patterned alternative ECM proteins and studied their efficacy for substrate-mediated AAV2 gene delivery. Currently, we have investigated collagen I, laminin, elastin, and poly-l-lysine (PLL). ECM proteins were adsorbed first, followed by the adsorption of AAV2. We find all proteins support cell adhesion and gene delivery. The integrated density of GFP expression as well as Hoechst staining were determined at 72 h post seeding. GFP fluorescence was normalized to nuclear staining and compared across both alkanethiol surface and protein (Figure 1). While all proteins and surfaces tested were successful, we observe that laminin pre-adsorption yields the highest gene delivery efficiency (Figures 1,2).

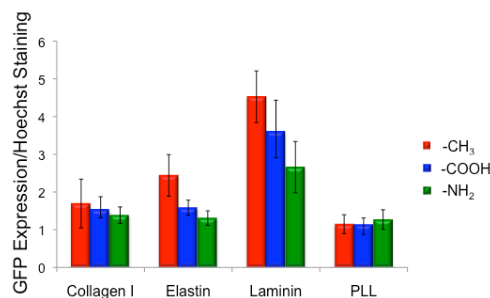

Figure 1. Normalized gene expression for AAV2-GFP reverse transduction from pre-adsorbed protein surfaces.

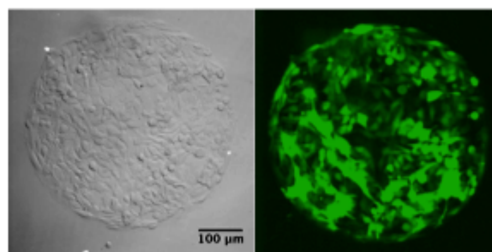

Figure 2. Cellular adhesion (DIC, left) and transduction (GFP expression, right) on pre-adsorbed laminin patterns.

Conclusions: Using microcontact printing and alkanethiol solutions, we have been able to successfully pattern both ECM proteins and viral gene delivery vectors. Using HeLa cells as a model, we have demonstrated that this platform technology is capable of facilitating efficient gene expression. Future work will involve the creation of more complex patterns to aid in the spatial organization of gene expression in developing cellular constructs.

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