Biomaterial-Mediated Retroviral Gene Transfer Using Self-Assembled Monolayers

Charles A. Gersbach[1], Joseph M. Le Doux[1], and Andrés J. García[2]
[1]Dept. of Biomedical Engineering, [2]Woodruff School of Mechanical Engineering, Georgia Inst. of Technology, Atlanta, GA

Statement of Purpose: Genetic engineering is essential for studying cell function and developing successful gene therapy techniques. However, inefficient and uncontrolled gene transfer by plasmid DNA or viral delivery has hampered the widespread efficacy of these approaches. Biomaterial-mediated gene transfer represents a promising strategy to address these limitations by immobilizing the gene carrier onto a biocompatible substrate[1]. This approach permits the control of gene transfer by co-localizing the cell adhesion substrate and the gene delivery vehicle[2]. These interactions must be carefully balanced to adequately immobilize the gene carrier to the biomaterial but also permit cellular uptake. Self-assembled monolayers (SAMs), such as alkanethiols on gold, are well-characterized biomaterials used to regulate adsorption of proteins and cell adhesion[3]. We hypothesized that SAMs of functionally-terminated alkanethiols presenting diverse surface chemistries can be used to control retroviral-mediated gene transfer to cells seeded onto these materials.

Methods: Tissue culture plastic (TC) was sequentially coated with titanium and gold by electron beam evaporation. SAMs were assembled by overnight incubation of gold-coated surfaces with the indicated alkanethiol (CH_3, COOH, or NH_2)[4]. SAMs were washed with PBS and incubated for 16 hrs in pTJ66 retroviral supernatant, which carries the eGFP transgene. Virus-coated surfaces were washed 2x with PBS, and NIH3T3 fibroblasts were seeded at 10,000 cells/cm^2. Cells were analyzed for eGFP transgene expression 3 days post-seeding by flow cytometry and fluorescence microscopy.

Results/Discussion: Fibroblasts seeded onto virus-coated surfaces exhibited SAM-dependent differences in transduction efficiency, as indicated by GFP expression (Fig. 1). Retroviral transduction was highest on NH_2 surfaces, which are positively charged at physiological pH (7.2). COOH surfaces, which are negatively charged in physiological solutions, showed modest GFP expression, and hydrophobic CH_3 surfaces displayed no transduction above background. These results are likely due to non-covalent (e.g. electrostatic) interactions of charged surfaces with viral particles.

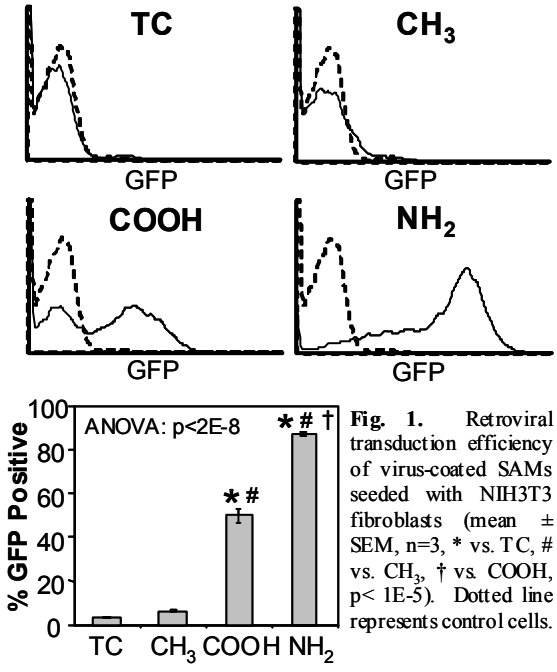

Fig. 1. Retroviral transduction efficiency of virus-coated SAMs seeded with NIH3T3 fibroblasts (mean ± SEM, n=3, * vs. TC, # vs. CH_3, † vs. COOH, p< 1E-5). Dotted line represents control cells.

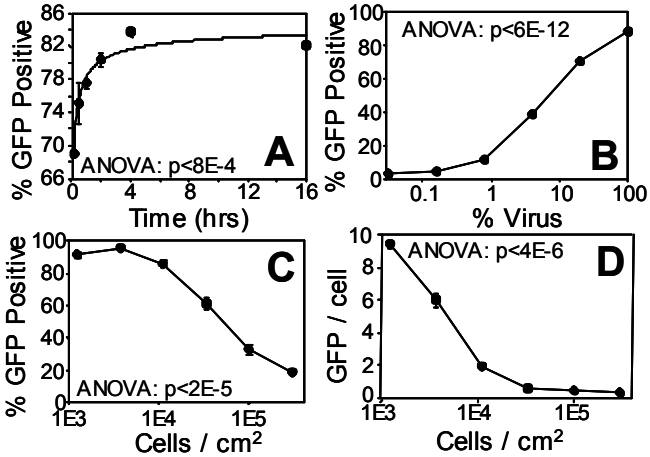

Fig. 2. GFP expression by NIH3T3 fibroblasts on NH_2 SAMs as a function of (A) virus-SAM incubation time (B) concentration of viral supernatant, and (C-D) cell seeding density.

Transduction by virus-coated NH_2 surfaces was further characterized by varying coating time, dilutions of viral supernatant with culture media, and cell seeding density. GFP expression saturated on surfaces coated for 4 hrs, but was still at significant levels (69%) on surfaces coated for only 15 minutes (Fig. 2A). Additionally, surfaces incubated with viral supernatant that was diluted 25-fold still transduced 40% of cells (Fig. 2B). Finally, the fraction of GFP-positive cells (Fig. 2C) and the level of GFP expression per cell (Fig. 2D) was modulated by cell seeding density, as cell division is required for retroviral integration. Finally, biomaterial-mediated viral gene transfer was spatially controlled by seeding cells onto virus-coated micropatterned surfaces of 10 μm NH_2-terminated lanes surrounded by nonfouling/nonadhesive triethylene glycol-functionalized domains (Fig. 3).

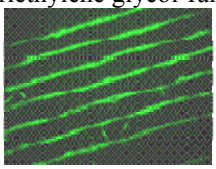

Fig. 3. eGFP expression by NIH3T3 fibroblasts seeded onto virus-coated micropatterned surfaces of 10 μm NH_2 lanes with EG_3 background

Conclusions: These results present a novel method for efficient viral gene transfer. This technique may assist in infecting cell types resistant to standard transduction schemes and spatially regulating gene transfer. This system has promising applications for engineering tissues, designing biomedical devices, and ex vivo gene therapy.

References: 1. Pannier et al. Mol. Ther. 10(1):19, 2004. 2. Bengali et al. Biotechnol. Bioeng. 90(3):290, 2005. 3. Mrksich et al. PNAS 93(20):10775, 1996. 4. Keselowsky et al. JBMR. 66(2):247, 2003.