Gene Delivery to Mouse Embryonic Stem Cells using Membrane Sandwich Electroporation

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Statement of Purpose: Over the past decade, the use of genetically modified primary embryonic stem (ES) cells has gained prominence as an attractive tool for a wide range of clinical applications. So far, this area is dominated by viral-mediated transduction of ES cells, which is very efficient but safety issues have hampered their clinical uses in humans. Non-viral methods are advancing as promising alternate approaches. However, low delivery efficiency of the therapeutic nucleic acid into the nucleus of the target cell is a significant obstacle in nonviral gene therapy. Previously, we developed a novel, much less invasive, and more efficient electroporation-based gene delivery method, called membrane sandwich electroporation (MSE) (Fei et al., 2007). In present work, we investigate MSE method for gene delivery to adherent, hard-to-transfect mouse embryonic stem cells (mESC). We use two types of support membrane substrates (gelatin coated polymer microarray or electrospun nanofibers) and quantify the results in terms of transfection efficiency and cell viability.

Methods: The CCE mESC line is used as cellular model. mESCs are maintained in the undifferentiated state and transfected with reporter gene pSEAP. pSEAP expression was quantified by alkaline phosphatase assay, and cell viability was measured using MTS assay.

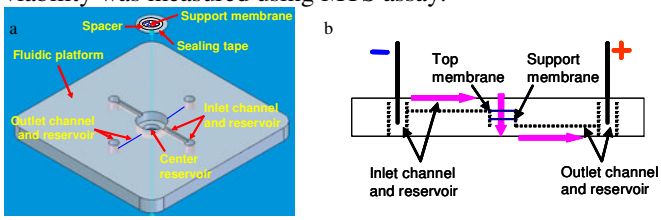

Figure 1. Experimental setup of (a) MSE platform and (b) schematic of DNA migration path.

The MSE set-up is shown in Fig 1. Two types of porous support membrane substrates are used for experiments: (1) a gelatin coated polymer membrane with Femtosecond laser drilled microarray: The small side of the pores is about 1 µm, and the large side is about 3 µm, (2) electrospun nanofibers (mean fiber diameter: 300-400 ìm, thickness: 100 ìm) of polycaprolactone (PCL) or PCL/Gelatin blends. The support membrane substrate is placed in the middle of a 1 cm diameter reservoir located at the center of the microfluidic device. The reservoir is connected to both the inlet (top) and the outlet (bottom) channels with a channel size of 500 µm in width and depth. A vacuum of (34 ± 3 KPa) is used to trap the cells on the porous support membrane substrates. Next, a polyethylene terephthalate (PET) track etch is placed over the immobilized cells with a spacer of ~10 µm between the two membranes. mES cells medium without serum is then loaded into the channels and the center reservoir, and the DNA sample is loaded into the inlet reservoir. Finally, EP is carried out, and pSEAP expression and cell viability are analyzed at 48 hr post-EP.

Results: In case of gelatin coated polymer microarray, pSEAP expression mediated by MSE has a significant improvement over bulk EP (Fig. 2a). Compared with 800V/cm used in bulk EP, a much lower field strength of 80 V/cm were applied in MSE, and thus mESCs experienced a ~ 75 percent survival rate in MSE, up from ~ 55 percent in bulk EP (Fig. 2b).

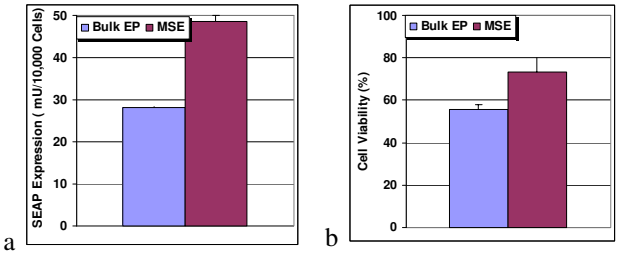

Figure 2. pSEAP Expression (a) and cell viability (b) at 48 hours post-EP by MSE on gelatin coated polymer microarray as compared to bulk EP.

In case of nanofiber substrates, two configurations, with or without top membrane were investigated. The use of PCL-gelatin or PCL nanofibers with top PET membrane also allowed for better confinement of DNA and cells resulting in higher SEAP expression levels (Fig. 3).

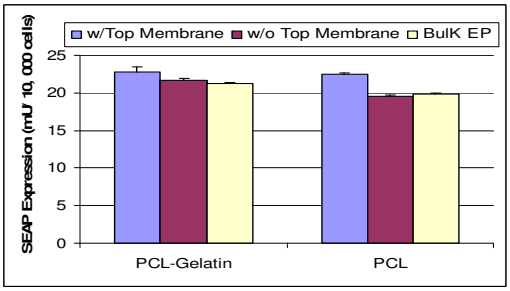

Figure 3. pSEAP Expression for electrospun PCL-Gelatin(a) and PCL(b) nanofibers at 48 hours post-EP by MSE as compared to bulk EP.

Conclusions: Using plasmid SEAP and CCE mESCs as model materials, our MSE method is able to provide better gene confinement near the cell surface to facilitate gene transport into the cells and thus shows significant improvement over transgene expression compared to current electroporation techniques. Gelatin coated polymer microarray or electrospun fibers enhance mESC adhesion resulting in better cell viability

References: Fei Z, et al. *Anal. Chem.* 2007; 79: 5719-22.