Supplementary Data

Magnetofection mediated transient Nanog overexpression enhances proliferation and myogenic differentiation of human hair follicle derived mesenchymal stem cells

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Optimal MF protocol for 293T cells (MF_{293T})

We optimized the efficiency of MF for gene delivery to 293T cells. First, we determined the most suitable MP:DNA ratio for MF under the following conditions: (1) formation of MP:DNA complexes in the presence of serum; (2) maximum magnetic field exposure time = 20 min; and (3) MP:DNA complex incubation time with cells = 4 hr. The amount of MP ranged from 0.25 to 1 μL, and the amount of EGFP expressing plasmid ranged from 0.25 to 2μg to form MP:DNA complexes at ratio of 1:1, 1:2 or 1:4, respectively (**Fig. S2A**). MF with MP:DNA ratio of 0.5μL:2μg resulted in the highest transfection efficiency of 94.48% ±7.33 EGFP+ cells and GFI of 130.76±8.5. This was similar to the results obtained with MP:DNA ratio of 1μL: 2μg but required less amount of MP. Therefore, this ratio was chosen for further optimizations.

Subsequently, we examined if the presence of serum during MF was beneficial for transfection efficiency. In the presence of serum, the transfection efficiency increased from 90.28±0.79% to 96.5±2.54% ($n=3$, $p<0.05$) of EGFP+ cells and GFI was enhanced from 115.36±12.89 to 176.5±30.70 ($n=3$ $p<0.05$) (**Fig. S2B**). Next we studied the effect of magnetic field exposure time on MF. Decreasing magnetic field exposure from 20 to 5min decreased the transfection efficiency and GFI to 69.19±7.12% EGFP+ cells and 40.00±4.7, respectively (**Fig. S2C**). Finally, we examined whether increasing the incubation time of MP:DNA complexes with cells after the magnetic field was withdrawn further enhanced MF efficiency. **Fig. S2D** indicates that both transfection efficiency and GFI increased with increasing incubation time from 86.96±3.00% (1 hr) to 99.6%±0.65 (20 hr) and from 71.75±1.16 (1 hr) to 507.96±51.22 (20 hr), respectively. Note that negligible cell death was observed during these experiments.

The optimized protocol was named MF_{293T} and it is as follows (**Fig. 3A**). Briefly,

cells were seeded at 2.5 x 10^5 cells/cm^2. The next day, MPs (0.5 µL) and DNA (2 µg) were mixed in DMEM (200 µL) for 20 min to allow MP:DNA complex formation and then added to the cells in 300 µL of growth medium. The magnetic field was applied for 20 min and fresh medium was changed after 4 hr. After one day of incubation, the transfection efficiency was measured using flow cytometry.

Supplementary figures

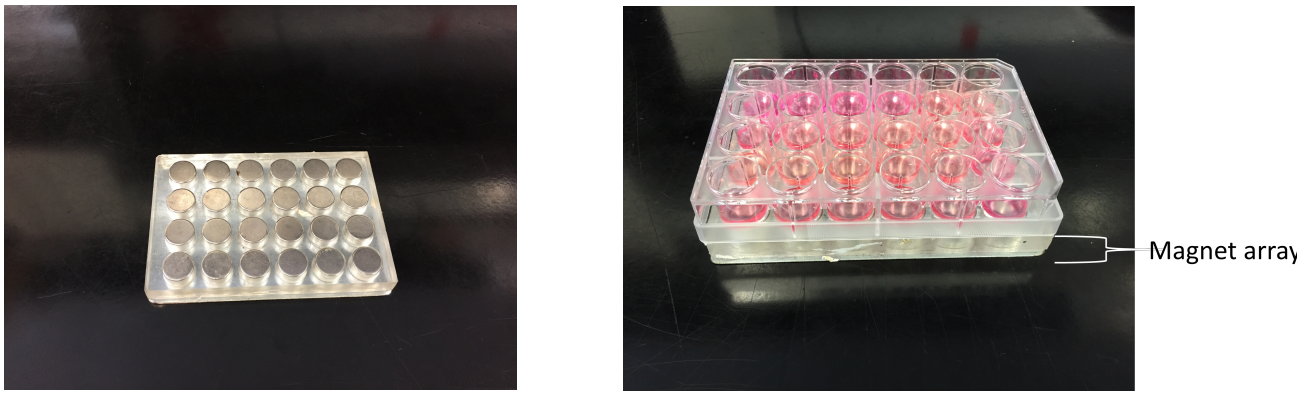

Figure S1: Image of our custom-made magnet array (left), which aligns precisely with a commercial available 24-well plate (right).

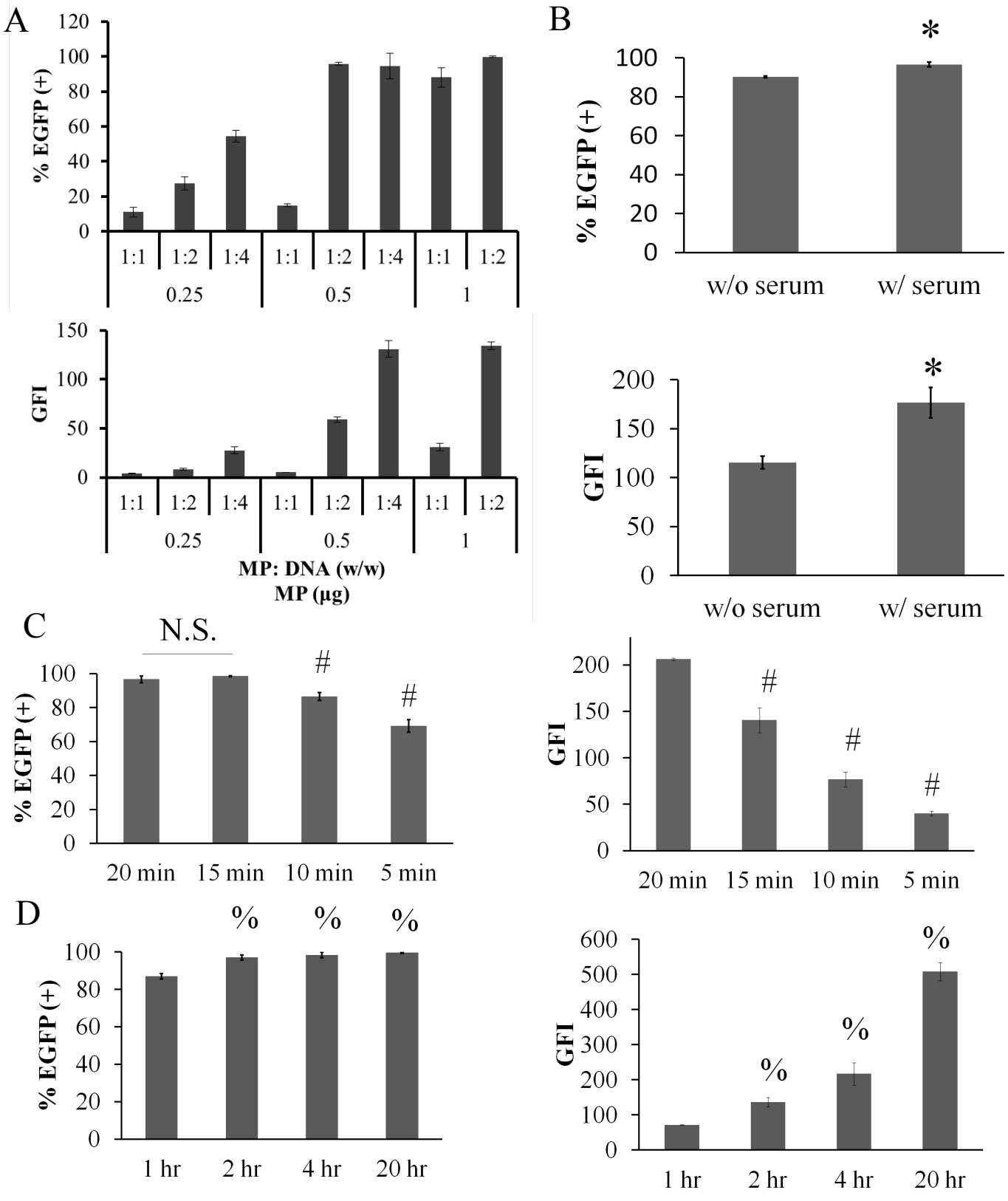

Figure S2: Optimization of MF in 293T cells.
The following parameters were investigated: (A) MP: DNA ratio; (B) presence of serum during MF; (C) magnetic field application time; and (D) MP:DNA complex incubation time

with target cells. All values are the mean ± SD of triplicate samples in a representative experiment (n=3). The symbol (*) denotes $p < 0.05$ between transfection w/o serum and w/ serum. The symbol (#) denotes $p < 0.05$ between 20 min and 10 or 5 min, respectively. The symbol (%) denotes $p < 0.05$ between 1 hr and 2, 4 or 20 hr, respectively.