INTRODUCTION:
Stem cells hold great promise for regenerative medicine and controlled stem cell differentiation is essential before their broad application. Knocking down genes inhibiting specific differentiation may promote the desired stem cell differentiation towards musculoskeletal pathway. RNA interference (RNAi) is a powerful gene-silencing mechanism and can be used to regulate stem cell fate by "turning-off" a target gene. Effective and safe delivery of RNAi therapeutics remains the key hurdle. In this study, a library of 10 novel lipid-like nanoparticles (lipidoids) was synthesized and screened in terms of high transfection efficiency and low cytotoxicity in human embryonic stem cell-derived cells (hESCs). As potential applications for bone tissue engineering, this lipidoid-based delivery system was examined to enhance stem cell differentiation towards bone pathway.

METHODS:
Lipidoid Synthesis: Lipidoids were synthesized and purified as previously reported. Briefly, lipidoids used in this study were synthesized by addition of acrylamide tails to amine monomers and stirred at 90°C for 7 days.

Biophysical Characterization: Particle size and ζ potential of the lipidoid/siRNA complexes were measured by using a ZetaPALS dynamic light scattering detector (Brookhaven). Atomic Force Microscopy experiments were conducted with Dimension 3100 series (Veeco) with non-contact mode Si cantilevers.

Cell Transfection: Human embryonic stem cell-derived cells (hESCs) were derived from hESC line H9 (WiCell) as previously described. Stem cells were seeded into 6-well plates (200,000 cells/well) and allowed to attach overnight. Cells were transfected with 800 ng/well of GAPDH siRNA complexed with lipidoid at lipidoid/siRNA ratios of 2.5:1 or 5:1 (wt/wt) to determine the optimum for transfection efficiency of hESCds. All experiments were performed in triplicate. The gene expression level of bone marker genes (cbfa1-1, ALP, Col I and osteocalcin).

Statistical Analysis: All experiments were performed in triplicate. The results were reported as means ± standard deviations. Statistical analysis was performed using analysis of variance (one way ANOVA) and statistical significance was set as p<0.05.

RESULTS:

DISCUSSION:
We have demonstrated lipidoids as a class of highly efficient material for siRNA delivery to hESCs. This study is the first demonstration of combinatorial approach to screen and optimize siRNA delivery nanoparticles to hESCs. Using lipidoid/GNAS siRNA nanoparticles, expression of GNAS (an inhibitor for cbfa1) was knocked down by 93% and significant enhanced osteogenesis was observed 14 days after a single transfection. These results suggest that lipidoids may serve as a powerful tool to direct stem cell differentiation towards musculoskeletal pathways through silencing of the associated inhibitors. The technology platform also holds promise for screening lipidoids-mediated siRNA delivery to other stem cell types and differentiation pathways.

ACKNOWLEDGMENTS: This work was supported by NIH DE016516 and EB000244. FY would also like to thank NIH for postdoctoral fellowship support.

REFERENCES: