Osteoblastic stimulation through gene silencing approach using mesoporous nanocarriers

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Statement of Purpose: Nano-sized carriers have become a special interest in drug delivery for tissue regenerative therapy. Among else, inorganic nanomaterials are easy to implement the size, shape and surface-functionality, and thus have the capacity for delivery systems of therapeutic molecules.[1] In particular, the mesoporous form has excellent loading capacity of drugs and genetic molecules. The gene silencing has shown immense potential for the disease treatment and tissue repair. Here we introduce the siRNA gene delivery system using mesoporous nanocarriers targeting bone repair.

Methods: Mesoporous and hollowed form of silica was prepared by replicating apatite nanorods, which was prepared by a hydrothermal procedure. The silica-apatite hybrid was produced by sol-gel reaction in tetraethyl orthosilcate. The hollow form was then prepared by removal of the inner apatite rod. The nanocarriers were characterized by transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FT-IR), and Brunauer Emmett Teller (BET). To form complexes with siRNA, nanocarriers were aminated with APTES. The siRNA was loaded in a range from 10 to 100 μg. Pre-osteoblastic cells were cultured with the siRNA-loaded nanocarriers at varying concentrations. The cell viability, intracellular uptake capacity, gene silencing efficiency, and bone-related gene expressions were investigated by MTS assay, confocal laser scanning microscopy (CLSM), fluorescence-activating cell sorting (FACS), and reverse transcriptase polymer chain reaction (RT-PCR).

Results: Rod-shaped nanocarriers with mesoporous structure were well obtained as revealed on TEM images. Based on the BET results, the surfaces area and pore volume of hollowed nanocarriers increased around 22-45%. The siRNA loading to the nanocarriers was confirmed, and the loading level was as high as ~30% of the nanocarriers. Cells were highly viable to the nanocarriers treated over a wide range (up to 160 μg). Confocal microscopic images of cells revealed the cellular uptake of nanocarriers as high as ~90%, which however, was not readily achieved with siRNA only. The transfected cells were shown to have efficient gene silencing of Plekho1, as analyzed by FACS. Furthermore, through the gene silencing, some key genes related with osteoblastic differentiation, including Runx2 and collagen type I, were substantially stimulated.

Conclusion: This study developed a novel gene delivery system for bone repair. Results demonstrated the effective cellular uptake, possible effects on gene silencing and stimulation of osteoblastic differentiation through the nanocarriers.

References: 1. Kwon S. Acta Biomater. 2014;10:1431-1442.