An Investigation of Porous PCL Scaffolds and Osteogenic and Angiogenic Inducing miRNAs for Bone Tissue Engineering

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Background
Trauma, infection, fracture, tumor resection, disease, or congenital abnormalities can cause bone defects. Small defects can self-regenerate, but larger ones or those with poor vascularized areas require intervention. Autografts involve transferring bone from one area to the damaged site. Although considered the current gold standard for treatment, this method is associated with limitations such as pain, infection risk, rejection and difficulty in finding the correct size, shape and volume of bone needed. Bone tissue engineering may be a promising alternative which combines multipotent cells, scaffolds, and bioactive factors to induce bone regeneration. The objectives of the study were to (1) investigate the effects of porogen amount, porogen size range, and coating of polycaprolactone (PCL) scaffolds on mesenchymal stem cell (MSC) and human umbilical vein endothelial cell (HUVEC) attachment and proliferation, (2) evaluate the osteogenic and angiogenic inducing properties of three variants of miRNA-26a.

Methods
Scaffolds were manufactured using a polymer casting-porogen leaching technique. Salt particles of specific sizes were used as porogens and dispersed within a solution of PCL and dichloromethane (DCM). The resulting scaffolds were coated with either gelatin or hyaluronic acid (HA) or cell media. The scaffolds were then seeded with either MSCs or HUVECs. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to determine cell attachment at day 1 and cell proliferation at day 7. MSCs were cultured and transfected with either a negative control, 26a 1-3p, 26a 2-3p, or 26a 5p miRNAs using Lipofectamine. Real-time-quantification polymerase chain reaction (RT-qPCR) and immunoblotting were performed 7 days after transfection to measure mRNA and protein levels of angiogenic and osteogenic markers, respectively.

Results
At Day 7, scaffolds seeded with MSCs at 80 % porosity and with a 300-500 μm porogen size range had significantly higher cell proliferation in comparison with those at 70 % and 90 % porosity and 106- 300 μm. For HUVEC cells, there was no effect of porosity and porogen size on cell proliferation. Scaffolds coated with gelatin had significantly higher cell proliferation in comparison to HA and uncoated. MSCs transfected with 26a miRNA variants had higher levels of osteogenic markers RUNX2, ALPL, and BGLAP and angiogenic marker VEGF in comparison to the negative control with miRNA 26a 2-3p having higher levels compared to the other variants. The immunoblotting assay determined MSCs transfected with 26a miRNA variants expressed RUNX2.

Conclusions.
In conclusion, we determined a scaffold at 80 % porosity, 300-500 um porogen size range, and coated with gelatin may be best suited for MSC and HUVEC attachment and proliferation. Furthermore, the three miRNA variants for miRNA-26a increased mRNA expression of osteogenic and angiogenic markers and RUNX protein levels. The utilization of tissue engineering through miRNA transfected MSCs combined with biocompatible biomaterials may serve as a promising approach for the treatment of bone defects.