INTRODUCTION: Angiogenesis plays an important role in regulating bone healing and successful bone tissue engineering. Adipose-derived stem cells (ADSCs) are a promising autologous cell source for angiogenesis due to their relative abundance, ease of isolation and potential to differentiate into vascular and mesenchymal lineages [1]. Genetic modification of stem cells to express angiogenic factors may further enhance the efficacy of stem cells for therapeutic angiogenesis. However, previous studies have relied on viral vectors, which is associated with safety concerns. Poly(β-amino esters) (PBAEs) is a family of hydrolytically biodegradable polymers that can condense DNA to form nanoparticles for safe and efficient gene transfection to human stem cells [2]. Here, we evaluate whether ADSC could serve as a delivery vehicle to target ischemia, and biomaterials-mediated gene delivery could be used to engineer ADSCs to overexpress angiogenic factors for therapeutic angiogenesis. Specifically, we developed biodegradable PBAE-DNA nanoparticles to program ADSCs to efficiently express CXC chemokine receptor 4 (CXCR4), an ischemia homing ligand, and/or vascular endothelial growth factor (VEGF), an angiogenic factor. The paracrine effects of non-viral engineered ADSCs on human umbilical vein endothelial cells (HUVEC) were evaluated in hypoxia condition in vitro, and therapeutic angiogenesis was examined in vivo in a murine hindlimb ischemia model (Fig. 1).

METHODS: ADSCs were transfected using biodegradable PBAE complexes with DNA plasmids p-EGFP-N1 or pBLAST49-hVEGF. Cells transfected using Lipofectamine 2000 was used as a control. Optimal transfection conditions were determined using flow cytometry, cell viability and VEGF ELISA. To determine the paracrine effects of VEGF-over-expressing ADSCs on HUVEC behavior, conditioned medium (CM) was collected from ADSCs 48 hours post-transfection and applied to HUVECs cultured under hypoxia condition. Outcomes were assessed by HUVEC cell viability and apoptosis under hypoxia condition (1% O₂), HUVEC migration, and tube formation assay. Passage 3 mouse ADSCs (GFP/Luciferase positive) were then transfected using optimized PBAE-mediated conditions. A murine hindlimb ischemia model was induced by ligating and removing the femoral artery. Cells (1×10⁶ cells per injection) were immediately injected intramuscularly into the ischemic limb. Three experimental groups were examined including mADSCs transfected using: 1) PBAE/CXCR4, 2) PBAE/VEGF, 3)PBAE/CXCR4/VEGF. ADSCs transfected using GFP was included as a control. Outcomes were evaluated using biodetection imaging (BLI), gene expression, limb physiology, and histology. Relative expression level of target genes was determined using ΔΔCt method, with GAPDH as the housekeeping gene.

RESULTS SECTION: PBAE-mediated gene delivery led to a 4-fold increase in transfection efficiency (23-24%) and VEGF production compared to positive control, Lipofectamine 2000 (5.3%). Under hypoxia, CM from VEGF-over-expressing ADSCs significantly reduced HUVEC apoptosis (13.7 ± 2.6%) compared with Lipofectamine (33.2 ± 2.4%, p < 0.05) and non-transfected ADSCs (39.0 ± 5.3%, p < 0.05). HUVEC preferentially migrated towards PBAE-VEGF transfected ADSC CM (16.8 ± 1.8%) versus non-transfected control ADSC CM (12.7 ± 1.3%, p < 0.05) and medium only (6.4 ± 1.9%, p < 0.05). PBAE/VEGF/ADSC CM also improved HUVEC tube formation (49.0 ± 4.4 branches) compared with Lipofectamine VEGF ADSC CM (32.7 ± 0.6 branches, p < 0.05) and non-transfected control ADSC CM (32.0 ± 1.8 branches, p < 0.05) (Fig. 2). When transplanted into hindlimb ischaemia mouse models, the CXCR4-ADSC group had the highest percentage of cells remaining at 24 hours (17.8 ± 5%) compared with CXCR4/VEGF-ADSC group (11.3 ± 1%, p < 0.05) and GFP control (3.0 ± 1%, p < 0.05). The CXCR4-ADSC group also demonstrated the longest survival and by day 15, CXCR4 or CXCR4/VEGF transfected ADSCs demonstrated higher BLI signals than the GFP control group. Twenty-eight days post-transplantation, CXCR4-ADSC treated group demonstrated 100% limb salvage, followed by groups treated by CXCR/VEGF-ADSC or VEGF-ADSC (75% limb salvage) (Fig. 3). Significant tissue damage was observed in the control group receiving GFP-ADSCs, with 50% limb loss and 50% with limb necrosis. Histology analysis at twenty-eight days post-transplantation showed significant necrosis in the PBS control group compared with ADSC transplanted groups. The CXCR4/VEGF-ADSC group demonstrated the least necrosis with the noted presence of capillaries. Quantitative gene expression of tissues harvested at day 30 showed that CXCR4/VEGF-ADSC treated group led to significantly increased expression of VEGF, SDF-1α, CXCR4.

DISCUSSION: Here we have demonstrated the promise of using genetically engineered ADSC for application in therapeutic angiogenesis, which is an important step to engineer critical size bone tissues. Our results suggest that co-delivery CXCR4/VEGF has the potential to improve ADSC homing to ischemia and enhance angiogenesis.

SIGNIFICANCE: Such platforms may be exploited for engineering viable bone tissues with clinically relevant dimensions for repairing large bony defects.

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