Synthetic Scaffold Functionalization by Adeno-Associated Virus Encoding BMP2 for Orthotopic Bone Defect Repair

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INTRODUCTION:  
Successful treatment of large bone defects with osteoinductive proteins generally requires high doses of recombinant protein that are costly and can cause inflammation or ectopic bone formation. Gene therapy using viral vectors offers a possibly superior protein delivery vehicle. The adeno-associated virus (AAV) may be the optimal virus for orthopaedic applications due to its host immune tolerance and ability to transduce a range of cells, infect dividing and non-dividing cells, and deliver long-term gene expression in some cell types. In vivo implantation of synthetic scaffolds lyophilized with AAV encoding the gene for bone morphogenetic protein 2 (BMP2) has been shown to cause ectopic bone formation. The objective of this study is to demonstrate for the first time that AAV-BMP2-functionalized synthetic scaffolds can be used to treat challenging orthotopic bone defects.

METHODS:  
2D and 3D In Vitro Studies: 20,000 human adult marrow-derived stem cells (hMSCs) or fetal amniotic fluid-derived stem cells (hAFS Cells) were seeded in 12-well plates and grown to confluence. Next, 0.5x10⁹ self-complimentary recombinant AAV-BMP2 (scAAV2.5-BMP2) or control AAV-Luciferase (AAV-Luc) particles were added per well in 10 µL media and gently agitated for AAV distribution. After 10 minutes 1 mL of media was added per well and plates were cultured in an incubator. After 75 minutes an additional 5 mL media was added per well. Scaffolds/stem cell constructs were cultured dynamically on a rocker plate in an incubator, and media supernates were collected twice weekly (n = 5 per cell type per AAV gene). For both 2D and 3D studies BMP2 was measured in collected supernates using an ELISA.

In Vivo Study: Bilateral 8mm critically-sized femoral defects were created in immunocompromised nude rats and stabilized by modular fixation plates. Defects were treated by implanting either acellular scaffolds lyophilized with scAAV2.5-BMP2 or AAV-Luc as described above (in vivo gene therapy – a variety of local host cells could be transduced) or scaffolds coated with the scAAV2.5-BMP2 or AAV-Luc. Cells were seeded on the scaffolds in 100 µL of media in 12-well plate wells. After 75 minutes an additional 5 mL media was added per well. Scaffold/stem cell constructs were cultured dynamically on a rocker plate in an incubator, and media supernates were collected twice weekly (n = 5 per cell type per AAV gene). For both 2D and 3D studies BMP2 was measured in collected supernates using an ELISA.

RESULTS:  
In Vitro: In both 2D and 3D experiments successful hMSC or hAFS Cells transduction was observed at 13-week-old ♀ immunocompromised nude rats and stabilized by modular fixation plates. Defects were treated by implanting either acellular scaffolds lyophilized with scAAV2.5-BMP2 or AAV-Luc as described above (in vivo gene therapy – a variety of local host cells could be transduced) or scaffolds coated with the scAAV2.5-BMP2 or AAV-Luc which had previously been seeded with 3x10⁶ hMSCs and cultured in vitro for 48 hours (in vitro gene therapy - specifically added cells, i.e. hMSCs, are transduced). Defect healing was assessed by X-rays and quantitative microcomputed tomography (µCT) scans 4 weeks after AAV transduction and BMP2 expression in different cell types, and possible loss of BMP2 expressed by hMSCs in culture prior to implantation. The experiment is ongoing and mineral formation will be monitored through at least four weeks for both stem cell types, which could lead to further mineral formation with time. In vivo results show that direct delivery of scAAV2.5-BMP2 coated scaffolds to the defect site leads to more defect bridging than by transducing hMSCs seeded on the scaffolds prior to implantation. This may be due to a variety of factors such as the amount of time that cells are directly exposed to the AAV, variability in scAAV2.5-BMP2 transduction and BMP2 expression in different cell types, and possible loss of BMP2 expressed by hMSCs in culture prior to implantation.

REFERENCES:  

ACKNOWLEDGEMENTS:  
Funding: NIH Grant NIH R01 AR051366  
Conflicts: Schwarz, EM - stock & research support from LAGeT Inc