INTRODUCTION

Approximately 10 million people in the US are diagnosed as osteoporotic, while an additional 34 million are classified as having low bone mass. Vertebral compression fractures (VCFs) are the most common fragility fractures accounting for approximately 700,000 injuries per year, twice the rate of hip fractures. Current treatment is mostly focused on prevention of VCFs mainly using new medicines such as Alendronate and Parathyroid Hormone.1 There are a few options of treatment when VCFs actually occur. Since open surgery involves morbidity and implant failure in the osteoporotic patient population, new minimally invasive solution are being developed. These methods include non-biological procedures involve injection of synthetic nonbiological and material that does not resorb and remains a permanent foreign-body fixture in the spine. Therefore there is a clear clinical need for a biological solution for vertebral bone repair. We have previously shown that BMP-modified adipose-derived stem cells (ASCs) are capable of inducing spinal fusion in vivo.2 In this study we hypothesized that direct injection of ASCs, transiently expressing BMP6, to a vertebral bone void defect would induce accelerated bone regeneration.

METHODS

Bone void defects were created in coccygeus vertebra of Nude rats. The spine was exposed and a surgical drill was used to create a 1mm in diameter and 2 mm in depth void. Porcine ASCs were isolated from adipose tissue and were labeled with lentiviral vector that encodes for two reporter genes, Luciferase (Luc) and GFP. (3) Labeled ASCs were further transiently transfected with a BMP6 plasmid using the electroporation-based system of nucleofection. (2) 24-hours later the cells were suspended in fibrin gel and injected into the bone void. The control group included defects injected with fibrin gel only. The repair process was monitored in vivo using µCT while cell survival was monitored using bioluminescent imaging every two weeks. The operated vertebrae were harvested after 12 weeks, and analyzed using ex vivo µCT, histology and immunohistochemistry against porcine vimentin.

RESULTS

In vivo bioluminescent imaging detected the luciferase-expressing cells at the implantation site for 12 weeks (Fig. 1). Since the ubiquitin promoter drives the Luc reporter gene in the injected cells, gene silencing does not occur over time. Therefore the gradual decline of the signal could indicate cell apoptosis that usually occur during MSC differentiation. The histological analysis of the defect site showed new bone formation that induced complete bone defect repair (Fig. 3). Porcine ASCs were detected in the site of new bone formation using immunohistochemical staining against porcine vimentin (Fig. 4).

DISSCUSSION

In this study we have shown the potential of directly injected, BMP-modified, ASCs to repair vertebral bone defects in a rat model. These results could pave the way to a novel approach for the biological treatment of traumatic and osteoporosis-related vertebral structures. Further studies in large animals should be performed in order to promote this stem cell-mediated strategy to the clinical practice.

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REFERENCES


Figure 1. Bioluminescent imaging quantification. Presented as average radiance emission per identical ROI. p<0.05; bars indicate SE.

Figure 2. µCT analysis. A at day 1 and at 8 weeks time point the defect was healed with ASC-BMP6. B 12-week time point of untreated and treated bone void.

Figure 3. Histological analysis. H&E stained slides are shown in x4, x10 and x20 magnifications.

Figure 4. Vertebral defect repair, immunohistochemical staining for porcine Vimentin, in newly formed bone (NB). Arrows indicate positive staining.