Introduction: Luciferase-mediated bioluminescent imaging is a powerful ex vivo technique assessing in vivo biological processes. In the luciferase reaction, photon emission results from the interaction of luciferase and the respective luciferin (coelenterazine) substrate. To measure the amount of photon production, and accordingly the amount of biological activity, a light sensitive apparatus known as a luminometer is employed. The data collected by the luminometer provides a quantified assessment of cell viability, which otherwise would occur via terminal tissue analysis. The purpose of this study is to evaluate Gaussia luciferase (Gluc) in urine specimens in monitoring implanted exogenous adipose-derived human stem cells (Ad-HMSCs, xenograft) viability in vivo and after exposure to rhBMP-2. Gluc, which is naturally secreted by the marine copepod G. princeps (Fig. 1), offers several advantages. Its activation can be monitored by assaying a few microliters of conditioned media without cell lysis. In addition, it can detect as few as 10 mammalian cells expressing it as well as being 2000-fold more sensitive than commonly used reporters such as luciferases from Renilla (fireflies) and the secreted alkaline phosphatase (SEAP). Ad-HMSCs can differentiate into osteoblasts, generating bone. rhBMP-2 is a “growth and differentiation” protein, known for its osteoinductive effects in humans and animals. The hypothesis is that higher photon measurement from Gluc urine specimens is associated with implanted Ad-HMSCs survival in vivo.

Materials and Methods: Cell Culture: Ad-HMSCs were isolated from human adipose tissue (lipoaspirated) using standard cell culture techniques. Then, the cell culture was expanded in Preadipocyte maintenance media. Finally, cells were transduced with a lentivirus expressing secreted Gaussia luciferase (Fig. 2).

Surgical Procedure: Posterolateral spinal fusion using Ad-HMSCs was performed in rats. Surgical exposure and decortication of the transverse processes were performed at the L4–L5 spinal level (facet joints left intact). Implants were placed in paraspinal muscle bed touching and spanning the transverse processes of L4–L5, on both the left and right sides (Fig. 3). Implants were 5x10⁶ Ad-HMSCs on Absorbable Collagen Sponge (ACS, 1.0 x 0.5 x 0.5 cm), 5x10⁶ Ad-HMSCs/ACS exposed to 0.003 mg/ml rhBMP-2, 5x10⁶ Ad-HMSCs/ACS+0.003 mg/ml rhBMP-2, or 0.003 mg/ml rhBMP-2/ACS only (no cells, control).

Urine Collection: Urine was collected through experimenter bladder expression daily over the first two weeks then every other day until sacrifice two months post-surgery. Urine Gluc expression was assessed via a luminometer in relative light units per second (RLU/s).

Euthanasia: Rats were euthanized 8 weeks after surgery.

Results: Results show average RLU’s highest from rats with 5x10⁶ Ad-HMSCs+0.003 mg/ml rhBMP-2/ACS, then 5x10⁶ Ad-HMSCs/ACS exposed to rhBMP-2, then 5x10⁶ Ad-HMSCs/ACS (no rhBMP-2), and lowest with only rhBMP-2/ACS (no cells, control) (p<0.05). Average RLU’s values agree with expected BMP-induced cell survival compared to no cell control conditions (rhBMP-2/ACS). Results demonstrate ex vivo reporter potential of Gluc to assess in vivo viability of implanted Ad-HMSCs in spinal tissue engineering (Fig. 4).

Discussion: Gaussia luciferase in urine may be a proxy ex vivo assay for quantifying the viability and growth of implanted Ad-HMSCs, exposed and unexposed to rhBMP-2, prior to terminal tissue harvest in spinal tissue engineering applications. Typically, Gaussia luciferase expression from stem cells is used as a tool to study the quantitative viability, differentiation, and proliferations of stem cells in a bioreactor. Fates of stem cells in vivo and cell proliferation counts show a strong association with expressed Gluc and GFP (2), thus providing a more direct observation of viability. The technique described herein employs a urine-based assay for frequent interval measurements in longitudinal studies and in doing so, avoids terminal tissue harvest.

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