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BONE MARROW MESENCHYMAL STROMAL CELLS DISPLAY A SMOOTH MUSCLE PHENOTYPE WHICH IS ENHANCED BY TGF(1: POTENTIAL USE TO TREAT SPHINCTERIC INCONTINENCE AND BLADDER WALL DEFECT

Hypothesis / aims of study

Marrow derived mesenchymal stromal cells (MSCs) possess mesenchymal plasticity making them ideal candidates for regenerative medicine applications for repair of damaged tissues. We propose that MSCs can be harvested, *ex vivo* expanded and subsequently utilized for uroregenerative purposes. In particular, to functionally replace *in vivo* the external sphincter and detrusor muscle. The initial step to attain this objective was to harvest rat bone marrow cells, isolate MSCs and ascertain their basal smooth muscle cell-like (SMC) phenotype, and develop a culture method to enhance *in vitro* differentiation into SMCs. The second step involved the injection of the MSCs into an animal model of stress urinary incontinence (SUI) and the measurement of their improvement using leak point pressure (LPP) recordings.

Study design, materials and methods

Femoral and tibial bones were collected from a normal female Sprague-Dawley (SD) rat and all nucleated marrow cells were isolated from bone marrow and resuspended in Dulbecco's Modified Eagle's Medium (DMEM) and 10% fetal bovine serum (FBS). Marrow cells were maintained at 37 C in a humidified atmosphere of 5% carbon dioxide. Non-adherent cells (hematopoietic cells) were discarded at first week. Adherent mesenchymal stem cells (MSCs) were subsequently passaged when they achieved 80% confluence. The phenotype of cultured MSCs (passage 6) was verified by flow cytometry for expression of cell surface markers and mesenchymal plasticity by use of differentiation media. For enhancement of SMC differentiation, MSC culture media was supplemented with 1ng/ml TGFβ1. Subsequent to a 14 day treatment, SMC phenotype was ascertained by immunofluorescence staining for smooth muscle cells markers (calponin, smooth muscle α-actin (SMA) and desmin). Western blots were performed on differentiated and undifferentiated MSC protein lysates for expression of calponin, SMA and desmin. We separated 17 female SD rats into 4 groups. Three rats were used as normal control and 14 underwent bilateral pudendal nerve transaction to create an animal model of SUI. 4 of these were SUI control rats, 4 underwent periurethral injection with Plasma Lyte (SUI placebo control) and 6 underwent periurethral injection of PKH 26 labeled MSCs. Four weeks after injection, conscious cystometrogram for each animal was performed and valsalva LPP was recorded.

Results

Tissue culture dish adherent rat MSCs have a doubling time of 48-56 hours. Cytometric analysis of these cells was performed. Cells were positive for the following cell surface antigens: CD44, CD73, CD90, RT1A, and negative for: CD31, CD45, and RT1B. Immunofluorescence microscopic analysis of MSCs revealed that undifferentiated and TGFβ1-treated rats MSCs express calponin. However, only the TGFβ1-treated MSC expressed SMA and desmin. The immunohistology results were validated using Western Blot analysis of cell lysates. The PKH 26 labelled MSCs injected periurethrally were found in situ and remained at the site of injection on pathology assessment. The LPP measurements (Fig.1) were statistically significantly different between SUI placebo controls (16,53±0,53 cmH2O) and SUI injected with MSCs (23,92±0,69 cmH2O) ($p<0.05$). (Fig.2)

Interpretation of results

Rat mesenchymal stromal cells express calponin. TGFβ1 treatment of rat MSCs skewed their phenotype towards that of true SMCs as demonstrated by upregulation of SMA and desmin expression. PKH 26 labelled MSCs injected periurethrally in an animal model of SUI significantly improved the valsalva LPP.

Concluding message

We propose that MSCs may be of use in uroregenerative medicine applications and future experiments will involve the use of scaffolds for *in vivo* MSC tissue replacement.

Figure 1. LPP measurements: A-Normal control, B-SUI control, C-SUI placebo injection, D- SUI treated with MSCs.

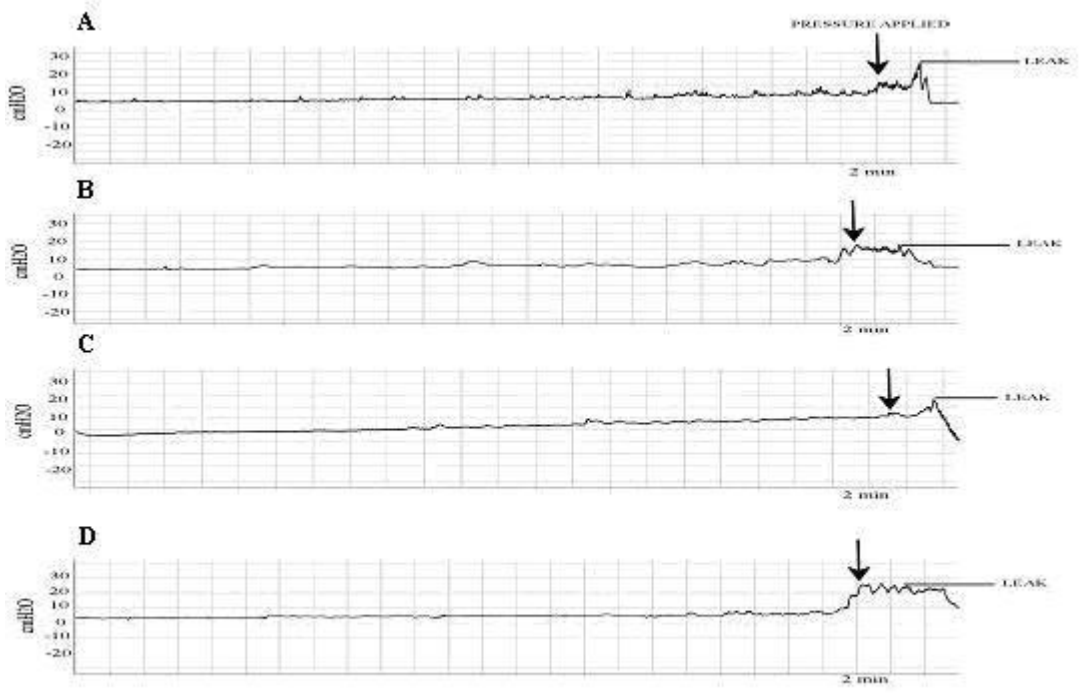
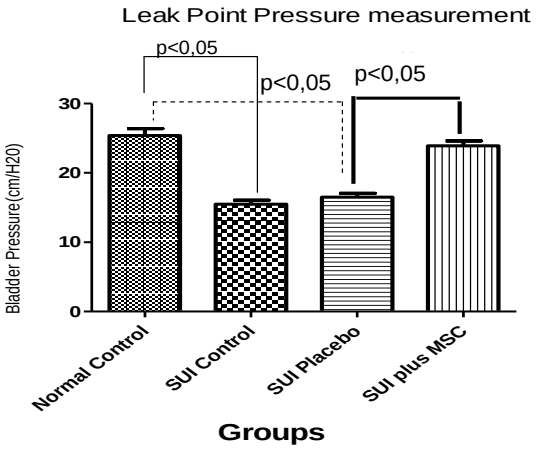


Figure 2. Data of LPP measurement



Normal Control	SUI control	SUI placebo	SUI plus MSC
27,1	17,2	16,4	22,5
23,7	14,6	17,8	23,9
25,4	14,9	15,2	21,5
	15,2	16,7	24,6
			24,7
			26,3
25.40 ± 0.9815	15.48 ± 0.5879	16.53 ± 0.5344	23.92 ± 0.6978

Specify source of funding or grant	**Division of Urology, Jewish General Hospital.**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**This study complies with Canadian Council on Animal Care guidelines and was approved by the McGill University Animal Care Committee.**