

BLADDER AUGMENTATION USING PLGA (POLYLACTIC-POLYGLYCOLIC ACID) SCAFFOLDS SEEDED WITH AUTOLOGOUS MESENCHYMAL STEM CELLS DERIVED FROM FAT AND SKELETAL MUSCLE: EXPERIMENTAL STUDY IN RABBITS.

Hypothesis / aims of study

In the urological field, the two most popular adult derived stem cell's sources for research are adipose tissue and skeletal muscle. The main advantages of these sources of stem cells are the large amount of available tissue and low harvesting morbidity. *In vitro* studies have been demonstrated the differentiation capacity of these cells. However, its *in vivo* application is limited by several issues. Constructs (scaffolds seeded with cells) have been developed for bladder reconstruction, but the results still remains unsatisfactory.

In the present study, we investigated the morphological and histological alterations, induced by PLGA scaffold seeded with autologous mesenchymal cells, derived from adipose tissue (ADSC) and skeletal muscle (MDSC), implanted on rabbit's bladder submucosa.

Study design, materials and methods

The animal ethical committee at our institution previously approved all procedures and the surgical procedures were performed following the good animal practices.

ADSCs were obtained from the left inguinal fat pad of 8 New Zealand male white rabbits. Briefly, the fat was washed with phosphate buffered saline to remove blood cells, and was finely minced using surgical scissors. The extra cellular matrix was digested for 30 min at 37°C with 0.075% collagenase and intermittent shaking. After filter in 100 µm strainer and centrifuged, we obtain the stromal vascular fraction. (1,2) The pellet was re-suspended in a culture medium (DMEM, 10% FBS, and 1% penicillin/streptomycin) and then plated in 100 mm² tissue culture flasks. MDSC were obtained from the left anterior tibial muscle (approximately 5 mm³) of 8 New Zealand male rabbits using the pre plated technique. (3)

ADSC and MDSC were left in culture until passage 2. Twelve hours before transplantation, the cells were rinsed with PBS and incubated with 1:200 dilution of dialkylcarbocyanine solution, a fluorescent cell membrane marker, (*Vybrant Dil*; Molecular Probes, Eugene, OR,) for 20 minutes at 37°C in accordance with the manufacturer's protocol. The labeled cells were harvested with 0.25% trypsin/1mM EDTA solution. To perform the autologous transplantation, it was prepared a cell suspension containing 1 X 10⁷ labeled cells in 30 µL of Hank's Balance Salt Solution to seed the cells on PLGA (polylactic-polyglycolic acid) scaffold just before bladder implantation. Two scaffolds were implanted on the bladder submucosa of each animal. One in the bladder's dome at the right side (without cells = control) and one in the bladder's dome at the left side (with cells). Eight animals receive ADSC seeded scaffold and eight animals received MDSC seeded scaffold. The animals were sacrificed at 4 and 8 weeks for bladder harvesting. The bladder was paraffin-embedded, sectioned in 4 µm slides and stained with H&E, Masson's Trychrome, IHC for alpha-smooth muscle actin. Localization of the Dil labeled cells in the host urethra was determined using an epifluorescence microscope, filtered for excitation/ emission at 546/590 nm to visualize the Dil label. The nuclei were counter-stained with DAPI.

Results

Four weeks after implantation, it was observed in the ADSC's and MDSC's seeded scaffolds the presence of neovascularization in the PLGA scaffold (figure 1), smooth muscle alpha-actin with minimum inflammatory response and Dil labeled cells (Figure2)

After 8 weeks, ADSC's and MDSC's seeded scaffolds induced small amount of collagen formation. It could be observed the presence of muscle spindles in the implanted area and Dil labeled cells localized around the muscle spindles (figure 3) Scaffolds without mesenchymal cells induced a large amount of collagen formation, and immunohistochemistry for alpha-actin and smooth muscle actin was negative.

Concluding message

PLGA scaffolds seeded with autologous ADSC or MDSC seems to be incorporated to the bladder wall without significant inflammatory response and lead to smooth muscle regeneration/migration and neovascularization, which is more pronounced 8 weeks after implant. It could be a future alternative to bladder augmentation.

References

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<i>Specify source of funding or grant</i>	Fundacao de Apoio a Pesquisa de Sao Paulo (FAPESP)
<i>Is this a clinical trial?</i>	No
<i>What were the subjects in the study?</i>	ANIMAL
<i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i>	Yes
<i>Name of ethics committee</i>	Federal University of Sao Paulo Ethical Comitee