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ADIPOSED DERIVED STEM CELLS SEEDED ON THE COLLAGEN MATRIX A NEW EXCITING OPTION FOR TISSUE ENGINEERING RECONSTRUCTION OF THE LOWER URINARY TRACT.

Hypothesis / aims of study

The urologic reconstructive surgeries are based on autologous organs or tissues transplantation. The harvesting of such tissues is always followed by some sort of morbidity. Skeletal muscle progenitors and autologous *in vitro* amplified smooth muscle cells has been proposed to repair the impaired tissues, but it still has some inconvenient. Adipose derived stem cells (ADSC) have described to have the ability to differentiate into adiposities, cartilage, bone, neuron and muscle. The ADSC can be used in the urological field as an easy source of cells to be used in urethral and ureteral reconstruction, bladder augmentation, urethral sphincter reconstruction and as injections to urinary incontinence and vesico-uretheral reflux. We describe our initial experience and technique of seeding rat ADSC on collagen based scaffolds (SIS, Cook Urological) that can be used in tissue engineering reconstructions of the lower urinary tract.

Study design, materials and methods

The 4 months old female rats were anesthetized using a combination of Ketamina and Xylazine to harvest the inguinal fat pad. The inguinal fat was processed as previously described (ref). Briefly, the fat was minced and washed extensively with 1X phosphate buffer saline (PBS). The extracellular matrix was digested with 0.075% collagenase at 37C for 30 minutes in constant agitation. The enzyme was inactivated with equal volume of Dubelco's modified Eagle media (DMEM) containing 10% fetal bovine serum (FBS). After centrifuge the solution at 250g for 10 min., it is obtained a high density processed lipoaspirate cell pellet. The pellet was ressuspend in DMEM with 10% FBS and an erythrocyte lysis buffer (0.16M NH4Cl) was added for 10 min, and then centrifuged again. The pellet was re-suspended and plated in 100mm dishes at the concentration of $1X10^5$. The cells were cultured in DMEM supplemented with 10% FBS (CM) until reach confluence. Once the cells reached the confluence they were seeded on commercial collagen based scaffold- SIS (0,5 x 1,0 cm) at the concentration of $1X10^6$. The cells were grown on these scaffolds and then evaluated regarding the distribution, viability and morphology.

Results

To obtain a cell coated SIS. The SIS was re-hydrated during 1 hour in sterile PBS and placed into a 100mm culture dishe. Using only 30 micro-litters of CM, $1X10^6$ cells were re-suspended and seeded on the SIS (0,5 x 1,0 cm). Two ml of CM were added to the dish, which just embedded the SIS, but did not cover it preventing cells floating. After 12 hours of incubation, the media was changed carefully to avoid cell floatation. With 24 hours the cells were attached to the SIS allowing addition of extra media, which were changed every other day. The cells stabilized and remained viable for 4 weeks, spreading over the SIS allowing differentiation and/or *in vivo* transplantation.

Interpretation of results

The ADSC have a unique characteristic of been a harvested in a expendable tissue with minimal morbidity and possessing a multi potent cell lineage differentiation ability. These cells have been demonstrated to be stable in culture, with low senescence rate, which allows great expansion in vitro and suggests that ADSC can very encouraging option for tissue engeneering. This study is to demonstrate the possible applicability of ADSC on urological reconstructions by seeding these cells on extra cellular matrix (ECM) or even in synthetic scaffolds (i.e.; PLA and PLGA). The ability to differentiate into different tissues of ADSC, will lead us to study the potential of ADSC on urological reconstructions by seeding this cells on scaffolds and transplant them before or after differentiation.

Concluding message

ADSCs are an easy source of cells that can be used for several modalities of urinary tract reconstruction providing an alternative source of cells for tissue engineering. The fat procurement process is simpler than other sources of mesenquimal stem cell and the capacity of these cells to differentiate into skeletal, smooth muscle, and nerve makes their use ideal for reconstruction of the urinary tract.