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Rodriguez L[1], Jack G[1], Zhang R[1], Turner A[2], Almeida F[2], Alfonso Z[1], Zuk P[1], Wu B[2]
1. The Geffen School of Medicine at UCLA, 2. UCLA

ADIPOSE DERIVED STEM CELLS FOR THE TISSUE ENGINEERING OF THE LOWER URINARY TRACT. IMPLICATIONS FOR THE TREATMENT OF STRESS URINARY INCONTINENCE AND BLADDER RECONSTRUCTION.

Hypothesis / aims of study

Loss of urethral support and atrophy of the intrinsic sphincter mechanism can result in SUI. Minimally invasive therapies for SUI offer injection of bulking materials into the periurethral tissues to improve urethral resistance. These therapies have had low success rates due to material migration, breakdown, and antigenicity. These bulking therapies only provide mass to the incontinent urethra, and they overlook the need for functional smooth muscle required to provide coaptation and contraction of the urethra. Bladder disease affects 400 million persons worldwide with gastrointestinal tissue being the gold standard when tissue is required for urinary diversion or augmentation; however, its use is associated with long-term complications. An easily obtainable source of healthy smooth muscle would be very useful in genitourinary tissue engineering. Ideally, these cells would be autologous. We have recently demonstrated that adipose derived stem cells (ADSC) are an autologous source of pluripotent cells. ADSCs are abundant in adipose tissue, amenable to harvesting under local anesthesia, and phenotypically similar to mesenchymal stem cells. In addition, ADSCs have been shown to differentiate into adipogenic, chrondrogenic, osteogenic, neurogenic and myogenic lineages. We investigated the ability of human ADSCs to be delivered to and survive within the bladder and urethral smooth muscle over extended periods of time. In addition, we investigated a tissue-engineered bladder generated from SMCs differentiated from ADSCs.

Study design, materials and methods

Lipoaspirate was acquired from female patients undergoing liposuction. The lipoaspirate was processed to yield a pluripotent population of adiposed derived stem cells (ADSC). For tissue delivery, the PLA cells were fluorescently labelled and suspended in Hank's balanced salt solution (HBSS). To assess ADSC viability within multiple animal models, Rnu athymic rats (n=8) and SCID mice (n=6) underwent laparotomy and injection of ADSCs into the bladder and urethra. An additional 8 rats underwent sham injection of HBSS alone. Experimental and control animals were sacrificed at 2, 4, 8, and 12 weeks post-injection, and the bladders and urethras were analyzed. In addition, the ADSCs were subsequently labelled with DiI, cultured in a media conducive to smooth muscle differentiation, and seeded at a density of 1x106 cells per cm^2 onto three dimensional composite grafts of electrospun 85:15% PLGA and PLGA porous sponges. Thirty adult rats underwent partial cystectomy and were divided into three groups: (1) Partial cystectomy alone, (2) Partial cystectomy and bladder augmentation with PLGA graft, (3) partial cystectomy and bladder augmentation with a 1 cm^2 patch of SM differentiated ADSC seeded engineered bladder. Urodyanmic testing was performed pre- and post-operatively at different time points on all animals. Bladders were harvested at 1, 2, and 4 weeks for histology.

Results

Self-regenerating, pluripotent ADSC cells were easily isolated from human adipose tissue. Evaluation at 2, 4, 8, and 12 weeks post-injection demonstrated ADSC cell viability and incorporation into the recipient smooth muscle. Eight weeks following injection, the ADSC cells demonstrated in vivo expression of alpha-smooth muscle actin, an early marker of smooth muscle differentiation.

ADSCs were negative for smooth muscle markers at the time of harvest. After 3 weeks in smooth muscle specific media, the ADSCs expressed myosin heavy chain (MHC), a late specific smooth muscle marker present only in contractile cells. The three dimensional PLGA grafts remained pliable and stable in culture for up to 4 weeks. The differentiated ADSCs adhered to the scaffold at 80% confluency and they maintained MHC expression for up to 4 weeks in vitro on the scaffold. Bladders augmented with the engineered scaffolds had

superior capacity and compliance compared to partial cystectomy. Only bladders with engineered cell seeded grafts showed development of smooth muscle layers similar to the native bladder. The engineered bladder walls had progressive urothelial coverage along the luminal surface at 1 and 2 weeks in vivo.

Interpretation of results

This is the first study to show incorporation of adipose derived stem cells into the smooth muscle of the urinary tract. In addition, for the first time, we showed that these cells have the ability to differentiate into smooth muscle in vivo. ADSCs remain viable up to 12 weeks in the lower urinary tract. In addition, we show the viability of using these cells for tissue engineering of the lower urinary tract. Tissue engineered bladders are achieved using ADSCs. The engineered bladders show normal compliance and capacity.

Concluding message

ADSC cells are an easily accessible source of pluripotent cells, making them ideal for tissue regeneration. Human ADSCs injected into the urinary tract show morphologic and phenotypic evidence of smooth muscle incorporation and differentiation with time. ADSCs may provide a feasible and cost-effective cell source for urinary tract reconstruction both for injectable forms of smooth muscle substitution in intrinsic sphincter deficiency as well as for tissue engineering of the bladder and lower urinary tract.

Bladder augmentation using scaffolds engineered from differentiated ADSCs results in improved bladder function compared to partial cystectomy. These engineered bladders have similar histology to the native tissue. ADSCs may offer an abundant source of smooth muscle for tissue engineering and bladder reconstruction in the future.

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