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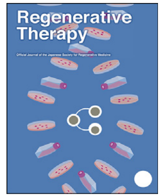
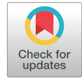


Original Article

Autologous and heterotopic transplantation of adipose stromal vascular fraction ameliorates stress urinary incontinence in rats with simulated childbirth trauma



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A B S T R A C T

Introduction: Autologous transplantation of adipose stromal vascular fraction (SVF) is a cost-effective and technically accessible option for cell therapy. Clinical study of SVF transplantation for male stress urinary incontinence (SUI) is underway, but the effectiveness remains unknown for female SUI, majority of which is caused by childbirth trauma.
Methods: Vaginal Distension (VD) rats were generated as *in vivo* model for female SUI. To quantitate the severity of SUI, leak point pressure (LPP) was measured by placing a bladder catheter. There was a characteristic waveform of LPP with two-peaks, and we counted the second peak as an LPP value. Adipose SVF was separated from inguinal fat and delivered into external urethral sphincter (EUS) through transperineal injection. LPP was measured 7 or 14 days after SVF transplantation. Tissue damage and collagen synthesis around the EUS were visualized by Masson's trichrome and eosin staining. Antibody against α-smooth muscle actin (α-SMA) was used to stain smooth muscle or activated stromal cells. Donor SVF cells were distinguished from recipient EUS tissue by tracking with *GFP* transgene.
Results: VD procedure decreased the frequency at which the normal LPP waveform appeared and lowered the LPP value. SVF injection normalized the waveform as well as the level of LPP. VD disrupted histological structure of EUS and SVF failed to differentiate into striatal muscles. Instead, SVF increased α-SMA positive cells and collagen synthesis but the phenomena depended on VD stimulus. GFP tracking indicated that the transplanted SVF cells persisted for four weeks and synthesized α-SMA protein simultaneously.
Conclusions: Autologous transplantation of adipose SVF displayed bulking effects through collagen synthesis. However, such heterotopic activation was dependent on tissue damage.
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1. Introduction

Stromal cells are heterogeneous cell population which support organ homeostasis [1]. During tissue damage, stromal cells are rapidly activated and intensively reorganized, to achieve the optimized tissue repair [1]. Adipose stromal vascular fraction (SVF) is one of the most abundant and accessible stromal cell sources and the feasibility for cell therapy has been widely studied [2]. Clinical study of SVF transplantation for male stress urinary incontinence (SUI) is ongoing [3–6], but the feasibility remains unknown for female SUI. In Japan, SUI affects around 13% of women over 40 years old, majority of which is caused by childbirth trauma [7]. In this study, we investigated whether autologous and heterotopic SVF injection has therapeutic benefits against SUI, using simulated childbirth trauma (vaginal distension) model rats.

Abbreviations: SVF, stromal vascular fraction; NPs, low-molecular-weight heparin/protamine micro/nanoparticles; SUI, stress urinary incontinence:; VD, vaginal distension; EUS, external urethral sphincter; LPP, leak point pressure; PNT, pudendal nerve transection.

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2. Methods

2.1. Animals

Virgin female rats of Sprague–Dawley (SD) strain at age 9–12 weeks weighing 240–320 g (CLEA Japan, Tokyo, Japan) were randomized into 6 groups for measuring leak point pressure (LPP): VD + No injection, Vaginal Distension (VD)-treated without Stromal Vascular Fraction (SVF) injection; VD + SVF, VD treated with SVF injection; VD + SVF + NPs, VD treated with SVF plus low-molecular-weight heparin/protamine nanoparticles (NPs) injection; Sham + no injection, sham-treated without SVF injection; Sham + SVF, sham-treated with SVF injection; Sham + SVF + NPs, sham-treated with SVF plus SVF + NPs injection. To track the donor cells after transplantation histologically, GFP-transgenic Lewis rats (LEW GFP; LEW-*Tg*$^{(CAG\text{-}EGFP)1Ys}$, a gift from Drs. Xiao-Kang Li and Hisashi Ueta) and its congenic Lewis rats (Japan SLC, Shizuoka, Japan) were used. We used LEW GFP and Lewis rats at age 10–12 weeks weighing 180–200 g bred in our animal experimentation facility. All animal experiments adhered to the *Guidelines for Animal Experimentation of Dokkyo Medical University*, with all efforts made to minimize the animal numbers and suffering.

2.2. Preparation of NPs

Low-molecular-weight heparin/protamine nanoparticles (NPs) were synthesized as described previously and used as extracellular scaffolds [8,9].

2.3. Preparation of SVF and SVF/NPs aggregates

Stromal vascular fraction (SVF) from autologous rats were prepared as previously described [10,11] with several modifications. Briefly, female SD rat's adipose tissue from the inguinal region was removed, minced, transferred to ‘C tubes’ (Miltenyi Biotec, Bergisch Gladbach, Germany) for enzymatic digestion. The solution contains 0.1% (weight/volume) collagenase type I (Wako Pure Chemical Industries, Osaka, Japan) and 0.2% (w/v) Dispase II (Thermo Fisher Scientific) in phosphate buffered saline (PBS). The minced tissue was kept at 37 °C for 1 h in shaking water bath (Taitec, Saitama, Japan) and mechanically stirred at every 10 min, using a MACS Dissociator (Miltenyi Biotec, installed software program “m_brain01-02”). The cell suspension was sieved through a 100 μm cell strainer (BD Falcon, Franklin Lakes, NJ, USA) and mature adipocytes and the debris were removed through the washing by PBS. Cells were subsequently centrifuged at 420 *g* for 5 min and resuspended in Dulbecco's Modified Eagle Medium (DMEM, Wako). The cell viability was determined with trypan blue exclusion method (Luna automated cell counter; Logos Biosystems, Anyang, South Korea). SVF (1.25×10^7 live cells per rat) were mixed with 100 μL of NPs (60 mg/mL) and incubated for 1 h at 37 °C to prepare SVF/NP aggregates.

2.4. Vaginal distension

We adopted the Vaginal Distension (VD) procedure as a tractable and reproducible rat model for human childbirth trauma [12]. Rats underwent VD under intraperitoneal ketamine (90 mg/kg) and xylazine (10 mg/kg) anesthesia as previously described [13,14]. A 10-Fr Foley balloon catheter (Create Medic, Yokohama, Japan) with the tip cut off was inserted into the vagina and the vagina orifice was closed with suture with 5/0 polypropylene (Prolene, Ethicon, New Brunswick, NJ, USA). The balloon was slowly distended with water to 3 mL [13]. After entire 4 h [15], the catheter was deflated. Also sham-distended rats were inserted the catheter into the vagina for 4 h but the balloon was not inflated.

2.5. Cell transplantation

Fig. 1a shows a schematic diagram of the method for the cell transplantation. After rats were anesthetized with ketamine and xylazine as described above, autologous SVF suspended in DMEM (2.5×10^6 cells; 20 μL per site) was injected in the 2, 4, 6, 8, and 10 o'clock positions around the urethral meatus using a 27-gauge needle (transperineal injection). The depth of injection (around 5 mm) was adjusted in advance so that the SVF spread around the external urethral sphincter (EUS) reproducibly.

2.6. Catheter implantation

On the day of LPP measurement, all rats underwent suprapubic bladder catheter implantation as previously described [16,17]. The rats were anesthetized as described above. A small incision was made in the bladder dome, and the polyethylene catheter (PE-50 tubing with a fire-flared tip) was inserted inside the bladder and secured with ligature through a bladder incision. The spinal cord was transected at the Th8–Th9 thoracic spinal level to prevent the spinobulbospinal voiding reflex, while maintaining urethral closure function intact [18].

2.7. Leak point pressure testing

Fig. 1b shows schematic diagram of LPP testing. The rats were placed into a sling-suit harness (Lomir Biomedical, Malone, NY, USA) and allowed to recover from the anesthesia for about 2 h, so that LPP testing was performed in an awake condition. The bladder was connected to a saline syringe via a bladder catheter. Intravesical pressure was monitored via a three-way stopcock which is connected to a pressure transducer. A data acquisition software recorded the pressure with sampling frequency at 10 Hz (Chart®, ADInstruments, Castle Hill, NSW, Australia) on a computer system equipped with an analog-to-digital converter (PowerLab®, ADInstruments, Dunedin, New Zealand). At the beginning the bladder was filled with saline until the initial leakage was observed from the urethral meatus. Hereafter, the ‘voiding (urination)’ could be easily distinguished from the ‘leakage’ because the pressure increase lasts longer during the voiding, while the leakage happened without such duration [17]. Subsequently saline was infused rapidly (3 mL/min) until a voiding was observed. The maximum pressure culminated the voiding was considered as LPP. We noted that the sham treated rats displayed ‘two peaks’ waveform for LPP (Fig. 1c). The second peak was larger than the first and when it was observed, it seemed that the animal was holding the voiding back (data not shown). We presume that the second peak reflects the major resistance for urinary continence and the sphincter is contributing to the peak. In fact, the level of the second peak gradually diminished over multiple testing, suggesting that the striatal muscle function declined due to overwork fatigue (data not shown). Importantly, VD treated rats frequently lost the second peak and numerical data was taken contingent upon observing such second peaks. The average of 4–12 consecutive LPPs was used as a data point of each animal. A 30-min interval was used for every three measurements to avoid the fatigue due to multiple testing.

2.8. Histology

Immediately after LPP testing, the animals were euthanized and the urethra and vagina were dissected *en bloc* at the level of the EUS for histological analysis. Tissues were immersion-fixed in 10 times-diluted neutral-buffered formalin (Original concentration is around 37% formaldehyde, Wako Pure Chemical Industries Ltd., Osaka, Japan). Subsequently, tissues embedded in paraffin, sectioned transversely (5 μm), and stained with Masson's trichrome (M-T) plus

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