

## Background and Aim

Radical hysterectomy to treat early-stage cervical cancer can yield good outcome; However, survivors may experience de novo lower urinary tract dysfunctions, which are due to damage of the pelvic nerve innervating the bladder during parametrial dissection. This study was conducted to demonstrate the effects of human amniotic fluid stem cells (hAFSCs) transplantation on bladder dysfunction and molecular changes in rats with bilateral pelvic nerve injuries (PNI).

## Introduction

Radical hysterectomy to treat early-stage cervical cancer can yield good outcome with 5-year overall survival over 80%. However, survivors may experience postoperative lower urinary tract dysfunctions due to damage of the pelvic nerve innervating the bladder during parametrial dissection in radical hysterectomy. To date, no effective treatment has been developed for bladder dysfunction after radical hysterectomy. Our published animal study demonstrated that transplanting human amniotic fluid stem cells into bladder wall could ameliorate bladder dysfunction after focal cerebral ischemia in rat. Until now, no study has been conducted to investigate the effect of hAFSCs on the bladder dysfunction caused by pelvic nerve damage in animal or human. This study wished to examine the effect of hAFSCs transplantation on bladder dysfunction induced by pelvic nerve transection (PNT).

## Methods and Materials

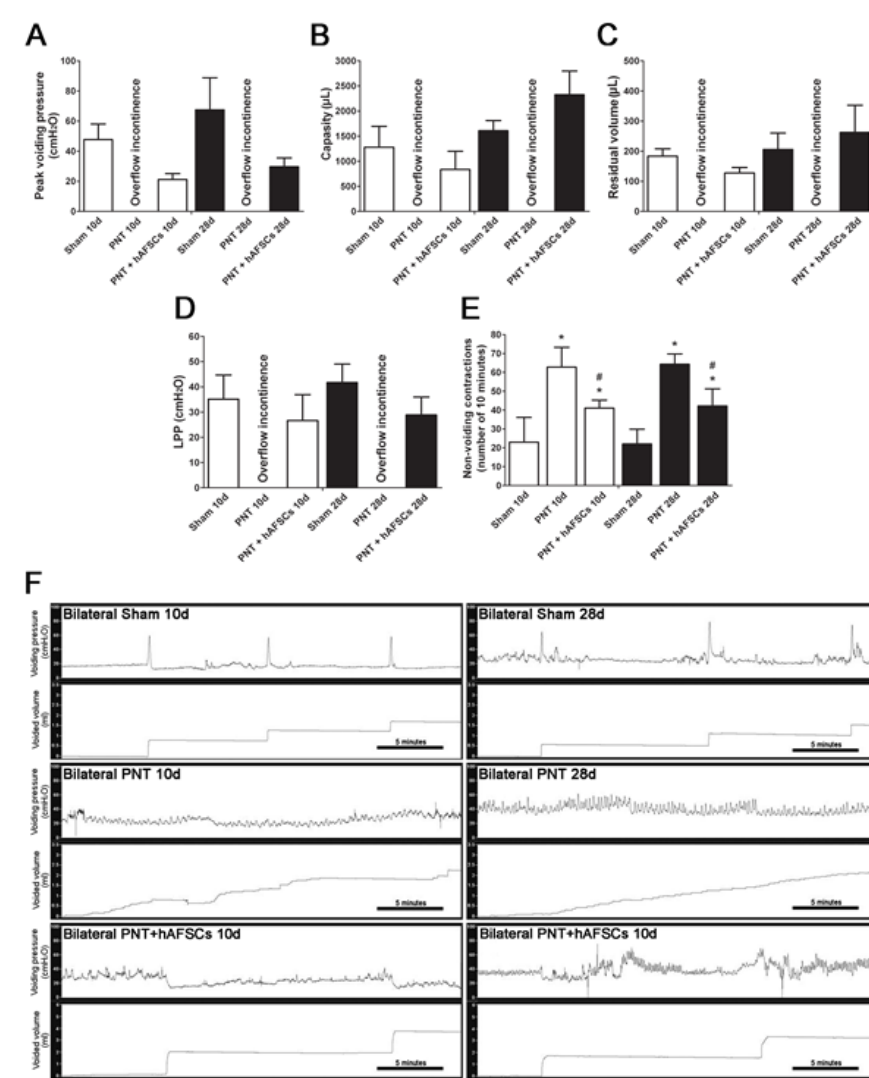
Sixty female adult rats were divided into sham group, bilateral PNI rats and bilateral PNI rats with injection of hAFSCs. The stem cells were obtained from freshly collected amniotic fluid by routine amniocentesis of healthy pregnant donors. Cells were cultured and incubated. Passage 4-6 hAFSCs were collected and prepared to a final concentration of  $1 \times 1000000$  cells/0.3 mL phosphate buffered saline. Because PNI interferes with bladder emptying, urine was manually expelled twice daily by gently depressing the lower abdomen. Cystometries at 10 and 28 days after sham or PNI were examined. Density of neurofilaments within bladder nerves and expressions of bladder protein gene product 9.5, growth-associated protein 43, nerve growth factor and p75 were studied using immunohistochemistry and real-time RT-PCR.

**Table 1.** Body weight and bladder weight in sham, PNTand PNT + hAFSCs.

Group (Mean ± SD)	Body weight (gm; Initial)	Body weight (gm; Final)	Bladder weight (mg)
Sham 10d	274.9 ± 18.7	277.4 ± 14.5	168.0 ± 28.6
PNT 10d	271.0 ± 9.9	274.7 ± 13.8	606.2 ± 66.6*
PNT + hAFSCs 10d	272.1 ± 12.2	276.6 ± 20.1	527.5 ± 79.4*
<i>P value</i>	0.874	0.958	<0.0001
Sham 28d	279.1 ± 20.2	284.0 ± 26.7	168.7 ± 48.7
PNT 28d	270.3 ± 8.5	283.8 ± 15.0	501.0 ± 63.9*
PNT + hAFSCs 28d	274.3 ± 11.9	289.2 ± 17.1	480.5 ± 79.5*
<i>P value</i>	0.534	0.829	<0.0001

## Results

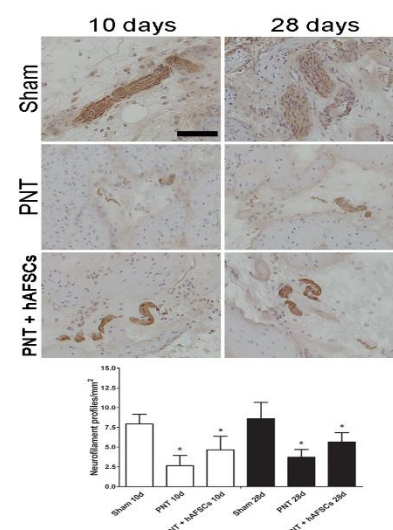
- Density of neurofilaments of bladder nerves are decreased at 10 and 28 days after PNI, but improve after hAFSCs transplantation.
- Bilateral PNI cause overflow urinary incontinence and increase number of non-voiding contraction. However, those cystometric parameters improve after hAFSCs transplantation.
- Immunoreactivities and mRNAs of protein gene product 9.5 and growth-associated protein 43 in PNI bladders decrease at 28 days after PNI. Transplantation of hAFSCs accelerate the recovery of protein gene product 9.5 to sham levels. Immunoreactivities and mRNAs of nerve growth factor and p75 significantly increase after PNI, but both decrease after hAFSCs transplantation.



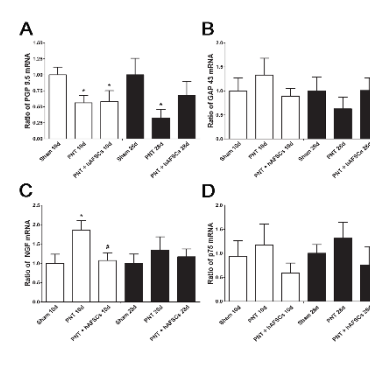
**Figure 1.** Urodynamic studies of all groups are presented

## Discussion

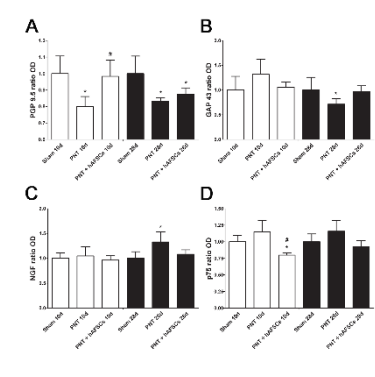
- Bilateral pelvic nerve crush caused hypertrophy of detrusor muscle and increased deposition of collagen and bilateral PNI caused muscle hyperplasia and hypertrophy in bladder wall with increased bladder weight in rats.
- Bilateral pelvic nerve crush increased bladder size, impaired detrusor contractility and decreased smooth muscle and autonomic innervation. Our study showed that bilateral PNI caused overflow incontinence and increased number of non-voiding contractions.



**Figure 2.** Representative images of neurofilaments.



**Figure 3.** Relative mRNA expressions.



**Figure 4.** Statistical analysis of the immunoreactivities.

## Conclusions

The current study demonstrates hAFSCs may facilitate the regeneration of pelvic nerves and improve bladder function in a rat model of unilateral or bilateral PNI. Moreover, as a non-invasive stem cell source, hAFSC is readily available from routine amniocenteses.

Bladder dysfunction induced by PNI can be improved after hAFSCs transplantation, and PGP9.5, GAP-43 and neurotrophins could be involved in the mechanisms of nerve regeneration in PNI bladder.

## References

- Plotti F, Angioli R, Zullo MA, et al. Update on urodynamic bladder dysfunctions after radical hysterectomy for cervical cancer. Crit Rev Oncol Hemat. 2011;80:323-329.
- Liang CC, Shaw SW, Huang YH, et al. Bladder Transplantation of Amniotic Fluid Stem Cell may Ameliorate Bladder Dysfunction After Focal Cerebral Ischemia in Rat. Stem Cells Transl Med. 2017;6:1227-1236.
- Kwon D, Minnery B, Kim Y, et al. Neurologic recovery and improved detrusor contractility using muscle-derived cells in rat model of unilateral pelvic nerve transection. Urology. 2005;65:1249-1253.