

RESEARCH ARTICLE

Bladder Recovery by Stem Cell Based Cell Therapy in the Bladder Dysfunction Induced by Spinal Cord Injury: Systematic Review and Meta-Analysis

Jae Heon Kim¹, Sung Ryul Shim², Seung Whan Doo¹, Won Jae Yang¹, Byung Wook Yoo³, Joyce Mary Kim⁴, Young Myoung Ko⁵, Eun Seop Song⁶, Ik Sung Lim⁷, Hong Jun Lee^{8*}, Yun Seob Song^{1*}

1 Department of Urology, Soonchunyang University Hospital, Soonchunhyang University School of Medicine, Seoul, Korea, **2** Institute for clinical molecular biology research, Soonchunyang University Hospital, Soonchunhyang University School of Medicine, Seoul, Korea, **3** Department of Family Medicine, Soonchunhyang University School of Medicine, Seoul, Korea, **4** International Clinic Center, Soonchunyang University Hospital, Seoul, Korea, **5** Department of Industrial and Management Engineering, Pohang University of Science and Technology, Pohang, Korea, **6** Department of Obstetrics and Gynecology, Inha University School of Medicine, Incheon, Korea, **7** Department of Industrial Management and Engineering, Namseoul University College of Engineering, Cheonan, Korea, **8** Medical Research Institute, Chung-Ang University College of Medicine, Seoul, Korea

* leejh71@gmail.com (HJL); yssong@schmc.ac.kr (YSS)



OPEN ACCESS

Citation: Kim JH, Shim SR, Doo SW, Yang WJ, Yoo BW, Kim JM, et al. (2015) Bladder Recovery by Stem Cell Based Cell Therapy in the Bladder Dysfunction Induced by Spinal Cord Injury: Systematic Review and Meta-Analysis. PLoS ONE 10(3): e0113491. doi:10.1371/journal.pone.0113491

Academic Editor: Naren L. Banik, Medical University of South Carolina, UNITED STATES

Received: April 6, 2014

Accepted: October 23, 2014

Published: March 17, 2015

Copyright: © 2015 Kim et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper.

Funding: This research was supported by Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-R1A1A2039317) and Soonchunhyang University Research Fund (20130616).

Competing Interests: The authors have declared no competing interests exist.

Abstract

Background

Bladder dysfunction induced by spinal cord injury (SCI) can become problematic and severely impair the quality of life. Preclinical studies of spinal cord injury have largely focused on the recovery of limb function while neglecting to investigate bladder recovery.

Objective

The present study was performed to investigate and review the effect of stem cell-based cell therapy on bladder recovery in SCI.

Methods

We conducted a meta-analysis of urodynamic findings of experimental trials that included studies of stem cell-based cell therapy in SCI. Relevant studies were searched using MEDLINE, EMBASE and Cochrane Library (January 1990 - December 2012). Final inclusion was determined by a urodynamic study involving detailed numerical values. Urodynamic parameters for analysis included voiding pressure, residual urine, bladder capacity and non-voiding contraction (NVC). Meta-analysis of the data, including findings from urodynamic studies, was performed using the Mantel-Haenszel method.

Results

A total of eight studies were included with a sample size of 224 subjects. The studies were divided into different subgroups by different models of SCI. After a stem cell-based cell therapy, voiding pressure ($-6.35, p < 0.00001, I^2 = 77\%$), NVC ($-3.58, p < 0.00001, I^2 = 82\%$), residual urine ($-0.24, p = 0.004, I^2 = 95\%$) showed overall significant improvement. Bladder capacity showed improvement after treatment only in the transection type ($-0.23, p = 0.0002, I^2 = 0\%$).

Conclusion

After stem cell-based cell therapy in SCI, partial bladder recovery including improvement of voiding pressure, NVC, and residual urine was demonstrated. Additional studies are needed to confirm the detailed mechanism and to obtain an ideal treatment strategy for bladder recovery.

Introduction

Worldwide, there are more than 130,000 new cases of traumatic spinal cord injury (SCI) annually, which is estimated to affect approximately 2.5 million people [1,2]. SCI affects people of all ages and the present lack of curative treatments result in life-long functional impairments that manifest as major physical disabilities [1]. Any recovery from functional loss that can occur depends on the level, nature and severity of the injury [3]. Severe injuries yield very poor outcomes and recovery is limited. Consequently, patients suffer from chronic paralysis and autonomic dysfunctions in the body segments below the site of injury [3]. In addition, SCI leads to disabling secondary complications, such as spasticity, bowel and bladder dysfunction, and development of chronic neuropathic pain [4].

Among the secondary complications caused by SCI, patients suffer from urological complications [5] that include severe lower urinary tract dysfunctions including overactive bladder and urinary retention, which result in increased bladder thickness and fibrosis [6,7]. The urinary bladder wall accumulates connective tissue and becomes fibrotic following SCI, which may adversely affect smooth muscle function and the micturition function resulting in a low compliance bladder with low capacity and high threshold pressure.

Although the significance of a neurogenic bladder caused by SCI has been documented in clinical studies associated with urinary tract infection and chronic renal failure [8], less focus has been directed to preclinical SCI studies, especially stem cell-based cell therapy. Most SCI studies with stem cell-based cell therapy have focused mainly on the motor limb function and sensory recovery. Impaired quality of life (QoL) from loss of limb function likely exceeds that of bladder dysfunction in the short term. However, in the long term, bladder dysfunction can become problematic and impair severely QoL [9]. Despite these concerns and obstacles, research on bladder recovery represents a promising area of research in neurourology. To date, similar to preclinical research, clinical trials have focused mainly on the recovery of motor and sensory function of limbs. Thus, limited evidence regarding bladder recovery exists [10,11].

In the present study, we investigated bladder recovery using stem cell-based cell therapy in bladder dysfunction induced by SCI employing meta-analysis of urodynamic findings in preclinical studies and highlighted the current status of stem cell-based cell therapy of bladder dysfunction in SCI with a systematic review.

Materials and Methods

Study design

A meta-analysis and systematic review were conducted according to predefined guidelines provided by the Cochrane Collaboration (2008).

Study selection

A comprehensive, multi-database electronic literature search was done to identify relevant research articles published between December 1990 and December 2012 involving varied SCI preclinical studies including stem cell-based cell therapy. The electronic search included the PubMed, Embase and Cochrane Library databases without language restrictions. The Medical Subject Headings (MeSH) and Emtree keywords included “spinal cord injuries”, “spinal injuries”, “stem cells”, “cells, cultured”, “stem cell transplantation” and “Rat”.

The studies were included if the described experiments included spinal surgery; the described interventions included administration of stem cell or other progenitor cells; the reported outcomes included urodynamic findings like voiding pressure, residual urine, non-voiding contraction (NVC) and bladder capacity; and rats were the experimental animal. The articles were excluded if the studies were unable to acquire full urodynamics data, involved non-randomized controlled animal trial of low quality, or involved combined treatment with medications.

Data collection and analysis

The initial screening with electronic databases to identify potential studies for inclusion based on title and abstract information was performed independently by two of the authors (Jae Heon Kim and Sung Ryl Shim). In cases of insufficient data, both authors reviewed the full text of the article for information and clarification. Final inclusion was determined by the senior authors (Hong Jun Lee and Yun Seob Song). References and data for each included study were carefully cross-checked to ensure no overlapping data was presented and to maintain the meta-analysis integrity.

Assessment of methodological quality

The included studies were assessed for risk of bias by the GRADE Working Group and a managing reviewer according to the Cochrane guidelines. The judgment of every item was low risk, unclear or high risk. Any disagreement regarding eligibility during the extraction was discussed and resolved. Six assessed items were random sequence generation, allocation concealment, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias.

Meta-analysis of urodynamic findings

Meta-analysis of the data including findings from urodynamic studies was performed using the Mantel-Haenszel method with Review Manager software (RevMan version 5.0; Cochrane Collaboration, Germany) and Comprehensive Meta-Analysis version 2.2 software (Biostat, Englewood, NJ, USA). All variables were continuous data, such as voiding pressure, residual urine, NVC and bladder capacity. Mean \pm standard deviation was used to calculate the weighted mean difference (WMD) and 95% confidence interval (CI).

Assessment of heterogeneity

Statistical heterogeneity was assessed using the I^2 value and the result of the chi-squared test. A $p < 0.1$ and $I^2 > 50\%$ were considered suggestive of statistical heterogeneity, prompting a

random effects modeling estimate. However, a non-significant chi-squared test result ($p \geq 0.1$ and $I^2 \leq 50\%$) indicated a lack of evidence for heterogeneity, but did not necessarily imply homogeneity, because there may have been insufficient power to detect heterogeneity.

Assessment of potential publication bias

Results of the comprehensive meta-analysis to examine any potential publication bias in the studies are shown as Funnel plots.

Results

Inclusion of studies

The initial search identified 1708 articles from the electronic databases (929 from Embase, 775 from Pubmed, four from Cochrane). After exclusion of 362 studies containing overlapping data or appearing in more than one database, and after screening the titles and abstracts, 918 studies that did not meet the inclusion criteria were further excluded. After intensive screening for detailed evaluation of the remaining 428 studies, 10 studies were eligible. Of these, two were excluded due to lack of specific urodynamic study information. Finally, eight studies that met all inclusion criteria were included. The eight studies consisted of 222 subjects (110 experimental and 112 controls). A detailed flow chart for selection is shown in Fig. 1. A systematic review of the 10 studies was conducted on the detailed experimental differences and expected mechanisms (Tables 1 and 2). In five studies [12–16], two different treatment subgroups were included. Therefore, the meta-analysis comprised a total of 13 trials (Table 3).

Risk of bias in the included studies

A summary of methodological domain assessment for each subject is detailed in Table 1. Only two studies demonstrated the possibility of bias in blinding of outcome assessment.

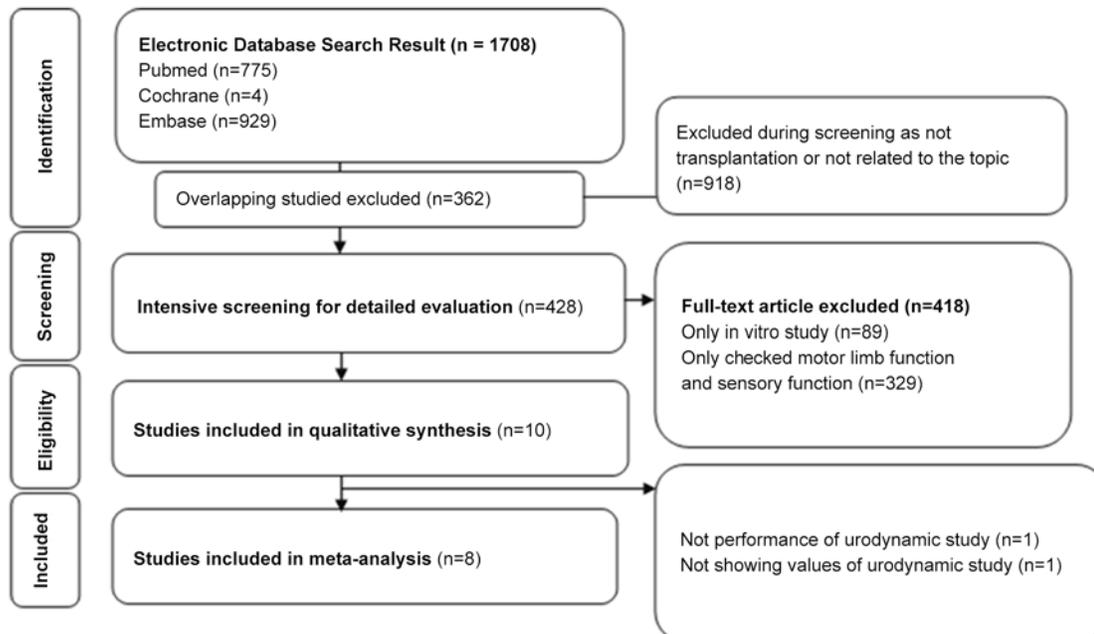


Fig 1. Method for study search and selection for inclusion.

doi:10.1371/journal.pone.0113491.g001

Table 1. Details of studies investigating stem cell-based cell therapy in spinal cord injury and bladder dysfunction.

Author	Injured level	Injury mechanism	Admitted cell	Route	Remarks	Expected mechanism	Graft survival confirmation
Hu, 2012 [17]	T8–9	Transection	BMSC, Bone marrow stromal cell	Intravenous	External urethral sphincter activity was checked, Coordination between bladder activity and EUS was checked	No sparing of descending modulatory pathway. Reorganization of synaptic connections. Formation of new pathways of voiding reflex	Immunohistochemistry of BrdU reactive cell
Jin, 2011 [12]	T9–10	Contusion	GRP, GDA	Direct injection	Daily records of bladder size and urine color, Human GRP and GDA did not promote axon growth	Neuroprotection, axonal sprouting, regeneration or myelination	HuNA (+) Immunofluorescence
Park, 2010 [15]	T8–9	Contusion	Human MSC	Direct injection	BDNF and NT-3 levels were checked by ELISA and RT-PCR at 28 and 56 days after MSC transplantation. No bladder recovery	Transplanted MSCs could reduce inflammatory process	Immunohistochemistry of anti-human nucleus antibody
Mitsui, 2011 [13]	T8–9	Contusion	NRP/GRP, AMPA/kinase blocker (NBQX)	Direct injection	Combined treatment using NRP/GRP and NBQX	Combined treatment have a potential of protection of host tissue and reduction of secondary injury	Not checked
Erdogan, 2013 [43]	T8–9	Transection, incomplete	Human umbilical cord MSC	Direct injection	Masson trichome stain used to demonstrate bladder morphology	Bladder structure change by reducing inflammation. Lamina propria layer change demonstrates reduced inflammation	Not checked
Telmeltas, 2009 [16]	T9–10	Hemisection	NRP/GRP, BMSC	Direct injection	Comparison of transplantation using NRP/GRP and BMSC	Neurogenesis by the capacity to grow new neurons from neural stem/progenitor cells	Direct count of cells
Mitsui, 2005 [18]	T8–9	Contusion	Fb-BDNF, Fb-NT3	Direct injection	Genetically modified fibroblast	Partial bladder recovery is mediated by the greater density of some descending fibers and decreased density of small caliber primary afferent projections by BDNF or NT3. Tissue sparing throughout the white matter and differentiation into neurons and glia	Direct cell confirmation by Nissl myelin staining. Immunoreactivity for RT-97
Neuhuber, 2008 [44]	T8–9	Contusion	NPC, Neural progenitor cell	Direct injection or lumbar puncture graft	Different route and method were compared. Direct injection vs lumbar puncture graft. Lumbar puncture graft showed localization at the dorsal aspect rather than entire injury site, which results in partial recovery of motor function		Direct cell confirmation by Nissl myelin staining. Alkaline phosphatase histochemistry
Mitsui, 2005 [14]	T8–9	Contusion	NRP/GRP	Direct injection	Examined the alpha-1A adrenergic antagonist effects. Tamsulosin showed positive effect in voiding pressure	Local protection provided by NRP/GRP results in increased sparing/sprouting of descending pathways. Modification of lumbosacral circuitry	Direct cell confirmation by Nissl myelin staining. Alkaline phosphatase histochemistry

(Continued)

Table 1. (Continued)

Author	Injured level	Injury mechanism	Admitted cell	Route	Remarks	Expected mechanism	Graft survival confirmation
Mitsui, 2003 [19]	T8–9	Contusion	EG6 immortalized neural stem cell	Direct injection	Observed difference in the degree of functional recovery between detrusor and external urethral sphincter: Functional recovery of somatic nerves is more easily accomplished by transplanted EG6 cells	Transplanted EG6 cells might differentiate and promote regeneration of injured spinal cord	Confirmation with BrdU immunohistochemistry

GRP, glial-restricted precursor; GDA, derived astrocytes; MSC, mesenchymal stem cell; BDNF: brain derived neurotrophic factor; NRP, neuronal-restricted precursor; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline; BMSC, bone marrow stromal cell; NT-3, neurotrophin-3; Fb, fibroblast; NPC

doi:10.1371/journal.pone.0113491.t001

Table 2. Study details including defense mechanisms, stability and plasticity of stem cells in stem cell-based cell therapy for SCI and bladder dysfunction.

Author	Migration	Host effects: spared host tissue, cyst and scar formation	Plasticity or projections	Differentiation	Functional recovery	Sensory recovery
Hu 2012 [17]	Yes	Not checked	L3–4	Not checked	Not checked	Not checked
Jin, 2011 [12]	Yes, long distance	Reduced cyst and scar formation. Reduced CSPG expression. Reduced glial scar formation	L6-S1, GRP Group showed reduced CGRP sprouting, GDA group showed higher 5-HT immunoreactivity	Differentiated into glial cells	No significant recovery in BBB test and grid test	Pain perception improved Thermal sensitivity (heat test). Mechanical sensitivity (Von Frey test)
Park, 2010 [15]	No checked	Not checked	BDNF and NT-3 levels showed no difference at thoracic and lumbar vertebrae L5-S1	Not differentiated	Limited recovery in chronic state in BBB test, coupling score, and ladder score	Not checked
Mitsui, 2011 [13]	No checked	Reduced cyst and cavity formation	5HT positive fibers showed higher density in both DL and DH. CGRP positive fibers in small diameter dorsal root afferents	Not checked	Recovery in BBB test	Not checked
Erdogan, 2013 [43]	Not checked	Not checked	Not checked	Not checked	Not checked	Not checked
Telmeltas, 2009 [16]	Not checked	Not checked	T9–10, E-NCAM (+), A2B5 (+)	Neuronal differentiation	Not checked	Not checked
Mitsui, 2005 [18]	Confirmed by Nissl-myelin staining	No difference in astrocytic scar formation	L6-S1, Afferent pathways using CGRP and VR-1. Suppression in transplanted group. No change in synaptic density. Descending pathways using 5HT, CRF, DβH. Positive for markers for neurons (MAP-2). Partially preserved or stimulated sprouting. No axonal growth by GAP-43	Partially neuronal regeneration. No axonal growth	No significant recovery in BBB test and recovery in grid test	No change in thermal sensitivity test

(Continued)

Table 2. (Continued)

Author	Migration	Host effects: spared host tissue, cyst and scar formation	Plasticity or projections	Differentiation	Functional recovery	Sensory recovery
Neuhuber, 2008 [44]	Some graft derived glial cells migrated away from the graft tissues Confirmed by Nissl myelin staining	Both group showed reduction in injury size and tissue sparing	L4–5, Oligodendrocytes (RIP) and astrocytes (GFAP)	Presence of neuronal and glial phenotypes. Neuronal differentiation	Intermediate recovery in BBB test	Not checked
Mitsui, 2005 [14]	Confirmed by Nissl myelin staining	Spared host tissue and cyst developed fewer	L6-S1, Afferent pathways: no differences in CGRP, VR-1, GAP43. Synaptophysin projections. NRP/GRP transplants inhibit dorsal root sprouting: CRF and DβH were diminished but lesser extent in transplant group. Sprouting or sparing of these pathways.	Neuronal differentiation Axonal neurofilaments with RT 97 antibody astrocyte differentiation with GFAP	Greater recovery of hindlimb function but similar results in grid test	Improvement in the thermal stimulus
Mitsui, 2003[19]	BrdU, Migration into white matter	Not checked	Not checked	Not checked	Not checked	Not checked

CSPG, Chondroitin sulfate proteoglycan; neuronal-restricted precursor; VR-1, vanilloid receptor type 1; CGRP, calcitonin gene-related peptide; DL, Dorsolateral nucleus; DH, Dorsal horn; BBB, Basso, Beattie, Bresnahan; 5-HT, serotonin; DβH, dopamine-β-hydroxylase; CRF, corticotropin releasing factor; GFAP, glial fibrillary acidic protein; GRP, glial-restricted precursor; GAP43, growth-associated protein 43

doi:10.1371/journal.pone.0113491.t002

These studies did not include the sham model. Overall, the risk of bias was considered to be low (Fig. 2).

Urodynamic findings

Detailed findings of the urodynamic studies are described in Table 3. Voiding pressure (11 trials), residual urine (9 trials), bladder capacity (10 trials) and NVC (8 trials) were analyzed. Study trials examining bladder weight, baseline pressure, voiding frequency and voiding efficiency were too small for analysis, and therefore those variables were excluded.

A total of 11 trials (n = 187; 94 experimental and 93 controls)[12–19] reported detailed data on voiding pressure. The WMD change of voiding pressure improvement from baseline was -6.35 (95% CI; -8.22, -4.48) (p<0.00001) (Fig. 3). Heterogeneity test showed p <0.00001 and Higgins' I² was 77%. Test of subgroup differences showed p = 0.18 and Higgins' I² was 43.6%.

A total of 9 trials in seven studies (n = 152; 79 experimental and 73 controls)[12–14,16–19] reported detailed data on residual urine. The WMD change of residual urine improvement from baseline was -0.24 (95% CI; -0.40, -0.07)(p = 0.004)(Fig. 4). Heterogeneity test showed p <0.00001 and Higgins' I² was 95%. Test of subgroup differences showed p <0.00001 and Higgins' I² was 98.9%. In transection model, residual urine showed significant improvement that the WMD change of residual urine in transection model was -0.88 (95% CI; -1.03, -0.73) (p<0.00001) (Fig. 4).

A total of 8 trials in six studies (n = 140; 72 experimental and 68 controls) [12–14,16–18] reported detailed data on NVC. The WMD change of NVC improvement from baseline was -3.58 (95% CI; -4.87, -2.29) (p<0.00001) (Fig. 5). Heterogeneity test showed p <0.00001 and

Table 3. Urodynamic study results of the eight enrolled studies.

Author	Admitted time	Cystometry time	Cystometric findings	Sham	Control	Transplanted group
Hu 2012 [17]	9 days after injury	28 days after transplantation		N = 6	N = 8	N = 8
			Voiding pressure	26.35±4.18	40.74±3.97	33.82±3.79*
			Residual urine (ml)	0.1±0.08	1.53±0.68	0.89±0.17*
			Bladder capacity (ml)	0.35±0.14	1.57±0.68	1.20±0.22
			Voiding efficiency (%)	70.38±10.10	2.78±1.46	26.25±6.93*
			NVC	0	9.67±3.83	2.13±1.13*
Jin, 2011 [12]	9 days after injury	8 weeks after transplantation		N = 4	N = 10	N = 9, Transplanted CRP
			Voiding pressure	15.58±2.58	42.32±4.07	36.21±1.72
			Residual urine	0±0	0.12±0.08	0.42±0.13
			Bladder capacity	0.33±0.03	0.60±0.20	1.04±0.10*
			NVC	0.25±0.15	16.36±5.57	6.25±0.79*
			Bladder weight: body weight ratio (g)	0.61±0.02	1.3±0.07	1.43±0.09
				N = 4	N = 10	N = 6, Transplanted GDA
			Voiding pressure	15.58±2.58	42.32±4.07	37.21±2.04
			Residual urine	0±0	0.12±0.08	0.13±0.08
			Bladder capacity	0.33±0.03	0.60±0.20	0.53±0.05
			NVC	0.25±0.15	16.36±5.57	19.25±1.70
			Bladder weight: body weight ratio (g)	0.61±0.02	1.3±0.07	1.38±0.08
			Park, 2010 [15]	9 days after injury	28 days and 56 days	
Voiding pressure	9.21±5.30	9.60±4.2				9.70±4.11
NVC (%)	43	36				44
Voiding frequency (time/min)	0.80±0.09	0.82±0.16				0.76±0.32
Bladder volume (mm ³)	1345.25 ±1206.27	1263.75 ±838.05				1279.57±1310.69
	N = 7	N = 9				N = 6, Transplanted (56 days)
Voiding pressure	9.00±5.77	8.11±7.39				10.33±4.50
NVC (%)	43	44				50
Voiding frequency (time/min)	0.79±0.11	0.82±0.19				0.92±0.07
Bladder volume (mm ³)	1874.47 ±1452.13	1925.99 ±1591.44				1594.45±703.36
Mitsui, 2011 [13]	9 days after injury	7 weeks after transplantation			N = 9	N = 10, Transplanted (NRP/GRP)
			Voiding pressure		36.1±2.7	27.9±2.0*
			Residual urine		0.16±0.04	0.14±0.08
			Bladder capacity		1.54±0.06	1.53±0.17
			NVC		7.0±1.0	3.4±0.9*
			Bladder weight (mg)		368.3±35.0	289.6±19.6*

(Continued)

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Mitsui 2003	+	+	+	?	+	+	+
Mitsui 2005a	+	+	+	+	+	+	+
Mitsui 2005b	+	+	+	+	+	+	-
Mitsui 2011	+	+	+	?	+	+	-
Telmeltas 2009_1	+	+	+	+	+	+	+
Telmeltas 2009_2	+	+	+	+	+	+	+
WBPark 2010_1	+	+	+	+	+	+	+
WBPark 2010_2	+	+	+	+	+	+	+
YHu 2012	+	+	+	+	+	+	+
YingJin 2011_1	+	+	+	+	+	+	+
YingJin 2011_2	+	+	+	+	+	+	+

Fig 2. Risk of bias summary. A review of the author’s judgments about each risk of bias item for each included study. “+” is “low risk”, “-” is “high risk”, “?” is “unclear”.

doi:10.1371/journal.pone.0113491.g002

Although contusion model showed no significant improvement after treatment ($p = 0.55$), transection model showed significant improvement after treatment ($p = 0.0002$) (Fig. 6).

Publication bias

Funnel plot analysis of 11 studies reporting voiding pressure is summarized in Fig. 7. Two studies positioned left of the funnel and two studies lie to the right. Begg and Mazumdar’s

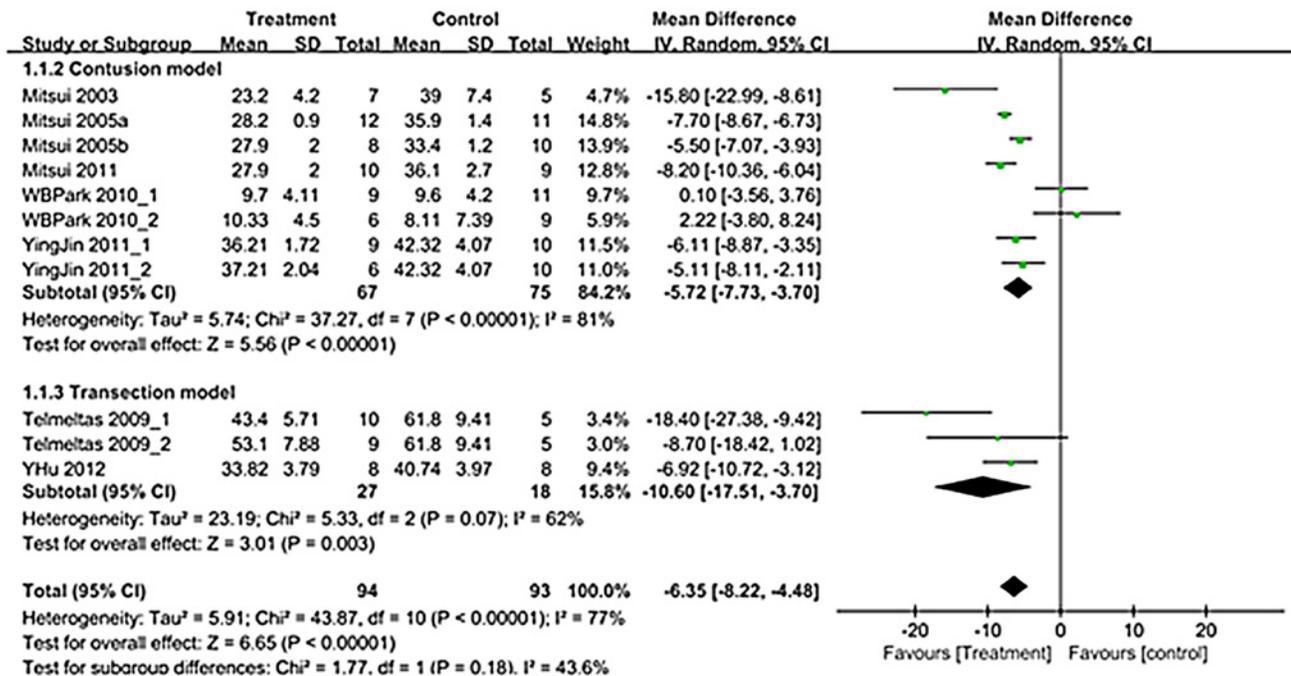


Fig 3. Forest plot diagram showing the effect of stem cell-based cell transplantation on voiding pressure. The black diamond signifies the mean difference is in favor of voiding pressure. The size of each square depends on the weight of each study. All data provided are for continuous outcomes.

doi:10.1371/journal.pone.0113491.g003

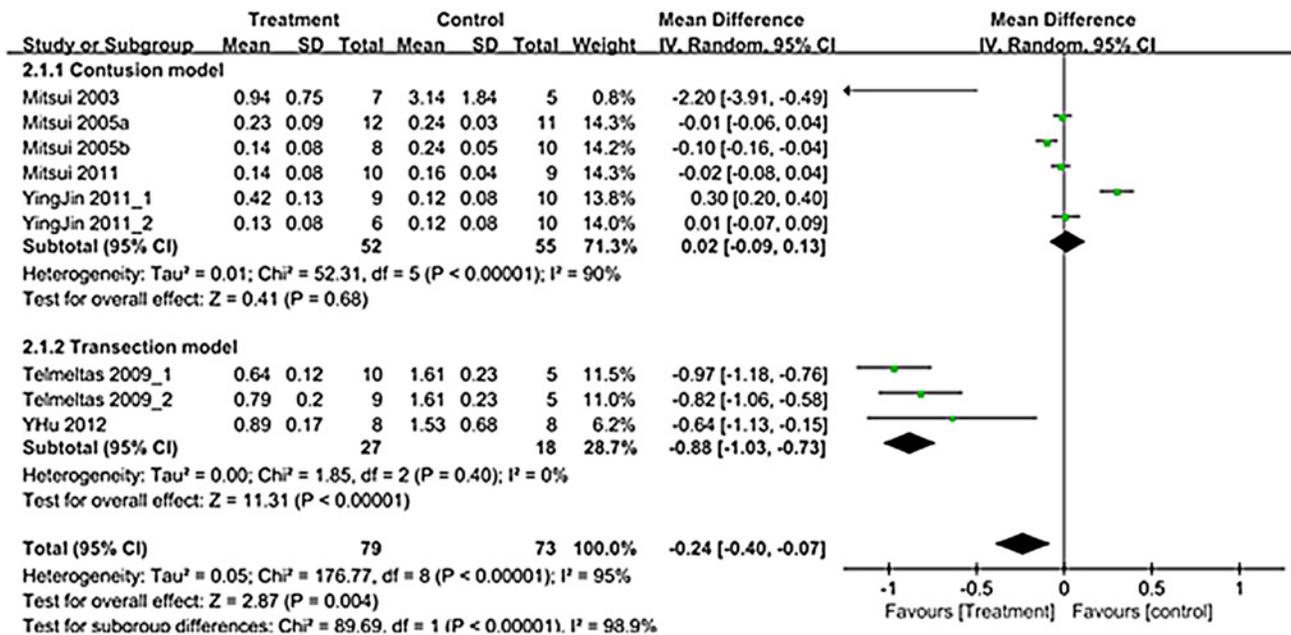


Fig 4. Forest plot diagram showing the effect of stem cell-based cell transplantation on residual urine. The black diamond signifies that the mean difference is in residual urine. The size of each square depends on the weight of each study. All data provided are for continuous outcomes.

doi:10.1371/journal.pone.0113491.g004

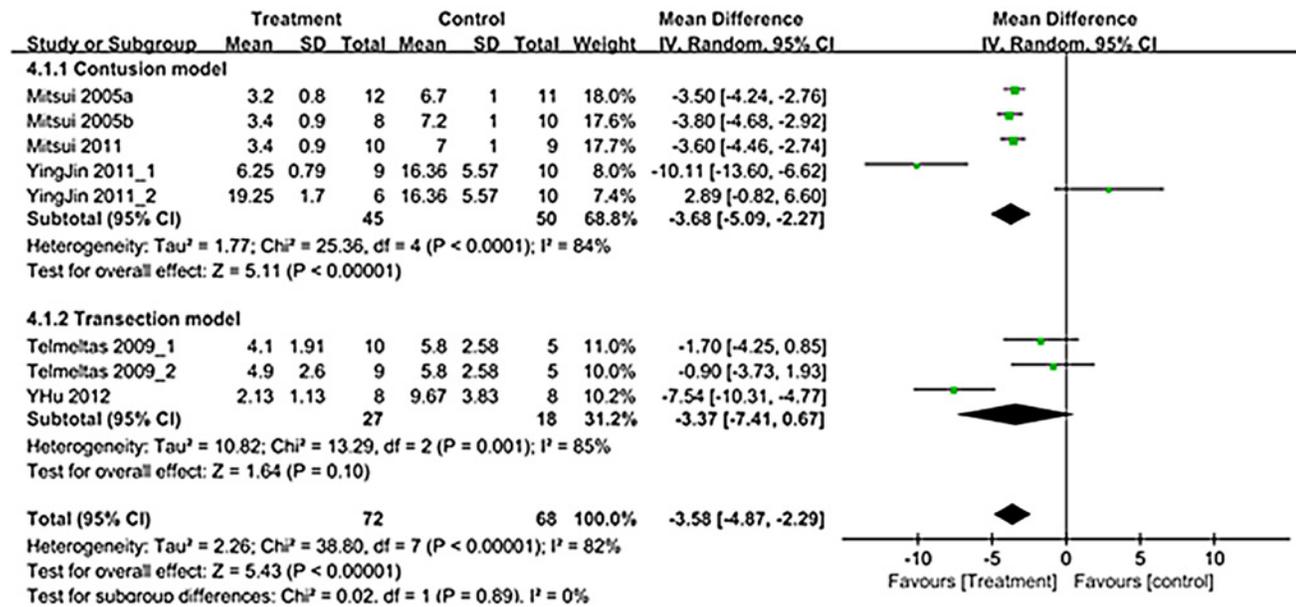


Fig 5. Forest plot diagram showing the effect of stem cell-based cell transplantation on non-voiding contraction (NVC). The black diamond signifies the mean difference is in favor of NVC. The size of each square depends on the weight of each study. All data provided are for continuous outcomes.

doi:10.1371/journal.pone.0113491.g005

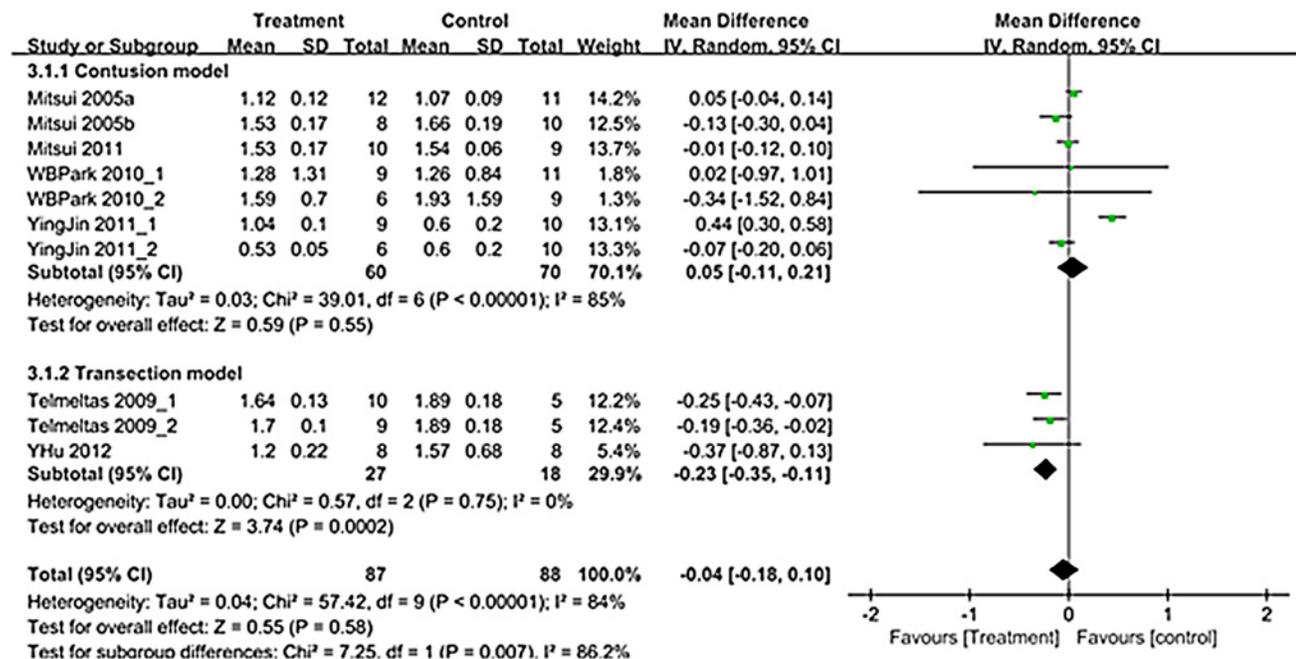


Fig 6. Forest plot diagram showing the effect of stem cell-based cell transplantation on bladder capacity. The black diamond signifies the mean difference is in favor of bladder capacity. The size of each square depends on the weight of each study. All data provided are for continuous outcomes.

doi:10.1371/journal.pone.0113491.g006

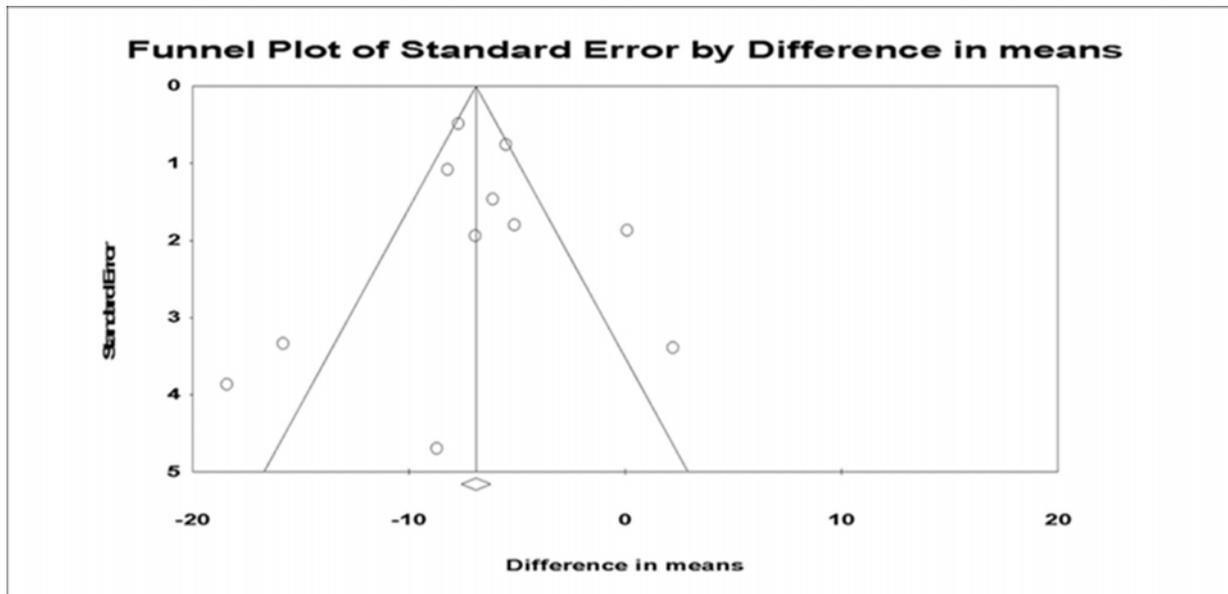


Fig 7. Funnel plot with pseudo 95% confidence limit.

doi:10.1371/journal.pone.0113491.g007

correlation was -0.091 (two-tailed $p = 0.697$). Egger's regression intercept was -0.230 (two-tailed $p = 0.842$). Thus, there was no evidence of publication bias in this meta-analysis.

Discussion

The use of neuroregeneration in stem cell-based cell therapy to correct bladder dysfunction is a logical and promising strategy. Spinobulbospinal pathways regulate and coordinate micturition reflexes. These pathways are located predominately in the dorsal and lateral columns of the spinal cord [20,21]. The contusion injury model, which is the most common SCI model, features the preferential destruction of the dorsal region of the spinal cord. This region is also destroyed in the transection model because injury to this region is necessary to study the spinal cord. The rat model of bladder dysfunction caused by SCI resembles the human condition [6,22,23].

Although complete recovery from SCI is impossible, several studies have demonstrated some functional recovery including limb function and sensory function. To date, very few studies have demonstrated bladder recovery utilizing urodynamic studies including cystometry (Table 3). This is the first study in which a detailed meta-analysis of urodynamic findings relevant to bladder function has been done.

Among the limitations of our study, the most important one is the several heterogeneities including types of SCI models and cell types (Tables 1–3). To control this heterogeneity, sub-analysis regarding the type of SCI model (contusion vs. transection) was done. Concerning the cell types, we did not analyze the subgroup analysis because the nature of the cells was similar and they shared the property of capability of regeneration into neuronal cells. Concerning the transplantation route, all studies adapted direct transplantation into the injured site except for one's study which had adopted the intravenous route. We did not conduct a subgroup analysis for this issue because of the small number of available studies and considering the homing and migration activity of stem cells.

Despite these heterogeneities, the timing of cell transplantation was the same (9 days after SCI), as was the timing of assessment of urodynamics (at least after 28 days following cell transplantation). Moreover, the injured site was similar with the thoracic vertebrae.

SCI weakens voluntary bladder and external urethral sphincter control in rats [21]. The main pathological micturition mechanism for bladder dysfunction following SCI is continuous co-contractions of the bladder and the sphincter leading to inefficient voiding and large residual urine volumes [24]. Continuous high bladder pressure and urine retention in SCI rats leads to complete deterioration of bladder compliance, function, infection and other lower urinary tract complications [25]. Augmented collagen deposition is considered to be an indicator of reduced bladder compliance, consistent with reports of experimental animal models with SCI [20,26]. SCI rats also exhibited frequent urination [7].

Presently, there was a significant improvement of voiding pressure after stem cell-based cell transplantation in the both contusion and transection models (Figs. 3). Residual urine and bladder capacity improved after treatment only in the transection model, and NVC improved after treatment only in the contusion model (Figs. 4–6).

Improved incidence of NVC during bladder filling has been described in all but two studies [15,19]. The main mechanism for this is that transplanted stem cells inhibit unmediated C-fiber sprouting from bladder afferents, which decreases the C-fiber bladder-to-bladder spinal micturition reflex in SCI rats [18,19].

It is logical to ascribe the late recovery to the parasympathetic system than somatic pathway system. Temeltas et al. [16] reported this issue in their recent study on transplantation of neuronal-glial restricted precursors or neural cells to rats with traumatic SCI [8], suggesting that recovery of function mediated by parasympathetic systems may be more difficult to accomplish than function mediated by somatic pathways [19].

The recovery of a micturition reflex is demonstrating by the descending projections, which diminish sprouting by C-fibers and by the provision of greater descending control over sensory transmission in the NVC [22,23,27,28]. Mitsui et al. [14] reported that the sprouting of primary afferents contributes to bladder dysfunction, specifically NVC, but the more nearly normal immunoreactivity can account for the diminished NVC in neural restricted precursor (NRP)/glial restricted precursor (GRP) rats.

Significant improvements were observed in voiding pressure and residual urine during micturition, which were attributable to alleviating sphincteric dysfunctions. The improvement of NVC and voiding pressure is due to the amelioration of dyssynergia between the bladder and urethral sphincter [19,29]. Attributable improvements in the neuronal circuitry include the central nervous system and the normalization of bladder tone. However, there was no decrease in the bladder capacity in most studies, compared with controls. Such an increase in the bladder capacity is thought to be caused by bladder over-distention during the reflex period by SCI itself [30]. The main mechanism of recovery of voiding function in the two different types of SCI is different. In the contusion model, the main mechanism involves the promotion of partial sparing of descending modulatory pathways that course through the lateral and ventral funiculi to the lumbosacral cord [14,18]. Spinal cord contusion interrupts axons from both ascending and descending tracts. A recent study demonstrated the effects of cell transplantation on general axon growth using neurofilament staining, and on regeneration of the afferent axons and descending serotonergic axons [31]. Voiding pressures were lower after transplantation of NRP/GRP, which indicates the amelioration of dyssynergia between the bladder and urethral sphincter [14,18,19,32]. In the transection model, the main mechanism is the reorganization of synaptic connections in dorsal gray commissure of L3–4, which is responsible for the genesis of the new voiding reflex pathways in SCI rats. Marson [33] and Lee et al. [34] found that neurons in the dorsal gray commissure activated by pudendal or pelvic nerve stimulation might integrate afferent signal neurons within multiple spinal segments.

Cell transplantation for the treatment of SCI has been studied extensively over the last two decades. A wide variety of stem cell-based cell types have been used based on their potential to

regenerate myelin, promote and guide axonal growth, bridge the site of injury or differentiate into neuronal or glial cells. Lepore et al. reported that NRP/GRP cells remain viable in the injured spinal cord, migrate outside of the injured region, differentiate into mature central nervous system phenotypes and support neuronal development [35].

Preclinical SCI studies have used neural stem cell/progenitor cells (NSCs/NPCs), umbilical cord blood derived cells, mesenchymal stem cells (MSCs), induced pluripotent cells (iPSCs), Schwann cells and olfactory ensheathing cells. Our enrolled studies measuring bladder dysfunction used NSCs/NPCs [12–14,16,18], MSCs or bone marrow stromal cells [15,17] and embryonic NSCs [19] (Table 1).

In the adult mammalian central nervous system, the genesis of new neurons has been documented, specifically in the subgranular layer of the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles [36]. In these regions, adult NPCs self-renew and become multipotent after *in vitro* transplantation into the central nervous system [37,38].

Despite significant advances in transplantation using NPCs, several disadvantages remain. These include inefficient tracking systems and moderate cell survival [39]. In addition, axonal regeneration by endogenous or exogenous NSCs may contribute to the formation of a glial scar [40]. Nonetheless, adult NSCs represent a safe, non-tumorigenic source of trophic support that may have merit for clinical application. Despite an absence of evidence of differentiation into neuronal cell types, several experimental studies have resulted in functional recovery in animals, supporting their potential use in cell therapy in SCI.

Among the enrolled studies, five used NPCs including neural restricted precursors, glial restricted precursors, human glial restricted progenitors, derived astrocytes and transgenically modified fibroblasts [18]. In SCI experimental models, MSCs were used less than NPCs, and MSCs were better in bladder outlet models. The main reason for this tendency is that NSCs/NPCs are considered more trophic and produce more favorable outcomes of neuronal regeneration after direct stem cell transplantation into the injured site.

MSCs were the first type of stem cells used to treat patients and, until recently, were the only stem cell type whose safety had been established [2]. Moreover, MSCs can transdifferentiate into neurons and glial cells [41], and can act in the physical guidance of neurofilament outgrowth in SCI [42]. However, among the enrolled studies, only two included human MSCs and two included bone marrow stromal cells [15,17] (Table 1). To date, most clinical trials of SCI have involved MSC-based cell therapy because of their relative ease of acquisition and safety [10]. To date, the role of MSCs in SCI is limited to the reduction of the inflammatory process within the injured spinal cord. Park et al. [15] could not demonstrate direct neuronal regeneration after transplantation of MSCs. However, clinical trials have shown limited bladder recovery using simple questionnaires and none included urodynamic studies.

Limitations in our study included heterogeneity. Lack of reproducibility resulting partly from differences in SCI models and treatment methods is the most critical issue. To date, no standard tool has been developed to check study quality, which makes meta-analysis of experimental research difficult.

Conclusions

The present meta-analysis and systematic review regarding bladder recovery after stem cell-based cell therapy in SCI demonstrates significant improvements in voiding pressure, residual urine and NVC. Considering the large volume of research on stem cell therapy in SCI preclinical studies, additional studies are necessary for improved understanding of bladder recovery.

Supporting Information

S1 PRISMA Checklist.
(DOC)

Acknowledgments

This research was supported by Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-R1A1A2039317) and Soonchunhyang University Research Fund (20130616).

Author Contributions

Conceived and designed the experiments: JHK HJL YSS. Performed the experiments: SWD WJY BWY JMK ESS. Analyzed the data: JHK SRS YMK. Contributed reagents/materials/analysis tools: SWD WJY BWY JMK ISL. Wrote the paper: JHK.

References

1. Adams M, Cavanagh JF (2004) International Campaign for Cures of Spinal Cord Injury Paralysis (ICCP): another step forward for spinal cord injury research. *Spinal Cord* 42: 273–280. PMID: [14968108](#)
2. Thuret S, Moon LD, Gage FH (2006) Therapeutic interventions after spinal cord injury. *Nat Rev Neurosci* 7: 628–643. PMID: [16858391](#)
3. Fawcett JW, Curt A, Steeves JD, Coleman WP, Tuszynski MH, et al. (2007) Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP panel: spontaneous recovery after spinal cord injury and statistical power needed for therapeutic clinical trials. *Spinal Cord* 45: 190–205. PMID: [17179973](#)
4. Yezierski RP (2005) Spinal cord injury: a model of central neuropathic pain. *Neurosignals* 14: 182–193. PMID: [16215301](#)
5. de Groat WC (1997) A neurologic basis for the overactive bladder. *Urology* 50: 36–52; discussion 53–36. PMID: [9426749](#)
6. Pikov V, Wrathall JR (2001) Coordination of the bladder detrusor and the external urethral sphincter in a rat model of spinal cord injury: effect of injury severity. *J Neurosci* 21: 559–569. PMID: [11160435](#)
7. Weld KJ, Graney MJ, Dmochowski RR (2000) Differences in bladder compliance with time and associations of bladder management with compliance in spinal cord injured patients. *J Urol* 163: 1228–1233. PMID: [10737503](#)
8. Thomas DG (1979) Genitourinary complications following spinal cord injury. *Practitioner* 223: 339–346. PMID: [514965](#)
9. Anderson KD (2004) Targeting recovery: priorities of the spinal cord-injured population. *J Neurotrauma* 21: 1371–1383. PMID: [15672628](#)
10. Geffner LF, Santacruz P, Izurieta M, Flor L, Maldonado B, et al. (2008) Administration of autologous bone marrow stem cells into spinal cord injury patients via multiple routes is safe and improves their quality of life: comprehensive case studies. *Cell Transplantation* 17: 1277–1293. PMID: [19364066](#)
11. Hernandez J, Torres-Espin A, Navarro X (2011) Adult stem cell transplants for spinal cord injury repair: current state in preclinical research. *Curr Stem Cell Res Ther* 6: 273–287. PMID: [21476980](#)
12. Jin Y, Neuhuber B, Singh A, Bouyer J, Lepore A, et al. (2011) Transplantation of human glial restricted progenitors and derived astrocytes into a contusion model of spinal cord injury. *Journal of Neurotrauma* 28: 579–594. doi: [10.1089/neu.2010.1626](#) PMID: [21222572](#)
13. Mitsui T, Neuhuber B, Fischer I (2011) Acute administration of AMPA/Kainate blocker combined with delayed transplantation of neural precursors improves lower urinary tract function in spinal injured rats. *Brain Research* 1418: 23–31. doi: [10.1016/j.brainres.2011.08.023](#) PMID: [21937028](#)
14. Mitsui T, Shumsky JS, Lepore AC, Murray M, Fischer I (2005) Transplantation of neuronal and glial restricted precursors into contused spinal cord improves bladder and motor functions, decreases thermal hypersensitivity, and modifies intraspinal circuitry. *J Neurosci* 25: 9624–9636. PMID: [16237167](#)

15. Park WB, Kim SY, Lee SH, Kim HW, Park JS, et al. (2010) The effect of mesenchymal stem cell transplantation on the recovery of bladder and hindlimb function after spinal cord contusion in rats. *BMC Neurosci* 11: 119. doi: [10.1186/1471-2202-11-119](https://doi.org/10.1186/1471-2202-11-119) PMID: [20846445](https://pubmed.ncbi.nlm.nih.gov/20846445/)
16. Temeltas G, Dagci T, Kurt F, Evren V, Tuglu I (2009) Bladder function recovery in rats with traumatic spinal cord injury after transplantation of neuronal-glial restricted precursors or bone marrow stromal cells. *J Urol* 181: 2774–2779. doi: [10.1016/j.juro.2009.01.093](https://doi.org/10.1016/j.juro.2009.01.093) PMID: [19375728](https://pubmed.ncbi.nlm.nih.gov/19375728/)
17. Hu Y, Liao LM, Ju YH, Fu G, Zhang HY, et al. (2012) Intravenously transplanted bone marrow stromal cells promote recovery of lower urinary tract function in rats with complete spinal cord injury. *Spinal Cord* 50: 202–207. doi: [10.1038/sc.2011.128](https://doi.org/10.1038/sc.2011.128) PMID: [22124344](https://pubmed.ncbi.nlm.nih.gov/22124344/)
18. Mitsui T, Fischer I, Shumsky JS, Murray M (2005) Transplants of fibroblasts expressing BDNF and NT-3 promote recovery of bladder and hindlimb function following spinal contusion injury in rats. *Exp Neurol* 194: 410–431. PMID: [16022868](https://pubmed.ncbi.nlm.nih.gov/16022868/)
19. Mitsui T, Kakizaki H, Tanaka H, Shibata T, Matsuoka I, et al. (2003) Immortalized neural stem cells transplanted into the injured spinal cord promote recovery of voiding function in the rat. *J Urol* 170: 1421–1425. PMID: [14501781](https://pubmed.ncbi.nlm.nih.gov/14501781/)
20. Nagatomi J, Gloeckner DC, Chancellor MB, DeGroat WC, Sacks MS (2004) Changes in the biaxial viscoelastic response of the urinary bladder following spinal cord injury. *Ann Biomed Eng* 32: 1409–1419. PMID: [15535058](https://pubmed.ncbi.nlm.nih.gov/15535058/)
21. Noto H, Roppolo JR, Steers WD, de Groat WC (1991) Electrophysiological analysis of the ascending and descending components of the micturition reflex pathway in the rat. *Brain Res* 549: 95–105. PMID: [1893257](https://pubmed.ncbi.nlm.nih.gov/1893257/)
22. Tsuruoka M, Maeda M, Nagasawa I, Inoue T (2004) Spinal pathways mediating coeruleospinal antinociception in the rat. *Neurosci Lett* 362: 236–239. PMID: [15158022](https://pubmed.ncbi.nlm.nih.gov/15158022/)
23. Ueyama T, Arakawa H, Mizuno N (1987) Central distribution of efferent and afferent components of the pudendal nerve in rat. *Anat Embryol (Berl)* 177: 37–49. PMID: [3439636](https://pubmed.ncbi.nlm.nih.gov/3439636/)
24. Barber MD, Brubaker L, Nygaard I, Wheeler TL 2nd, Schaffer J, et al. (2009) Defining success after surgery for pelvic organ prolapse. *Obstet Gynecol* 114: 600–609. doi: [10.1097/AOG.0b013e3181b2b1ae](https://doi.org/10.1097/AOG.0b013e3181b2b1ae) PMID: [19701041](https://pubmed.ncbi.nlm.nih.gov/19701041/)
25. Yoshiyama M, Nezu FM, Yokoyama O, de Groat WC, Chancellor MB (1999) Changes in micturition after spinal cord injury in conscious rats. *Urology* 54: 929–933. PMID: [10565763](https://pubmed.ncbi.nlm.nih.gov/10565763/)
26. Uvelius B, Mattiasson A (1984) Collagen content in the rat urinary bladder subjected to infravesical outflow obstruction. *J Urol* 132: 587–590. PMID: [6471202](https://pubmed.ncbi.nlm.nih.gov/6471202/)
27. Athwal BS, Berkley KJ, Hussain I, Brennan A, Craggs M, et al. (2001) Brain responses to changes in bladder volume and urge to void in healthy men. *Brain* 124: 369–377. PMID: [11157564](https://pubmed.ncbi.nlm.nih.gov/11157564/)
28. Suzuki R, Morcuende S, Webber M, Hunt SP, Dickenson AH (2002) Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. *Nat Neurosci* 5: 1319–1326. PMID: [12402039](https://pubmed.ncbi.nlm.nih.gov/12402039/)
29. Seki S, Sasaki K, Fraser MO, Igawa Y, Nishizawa O, et al. (2002) Immunoneutralization of nerve growth factor in lumbosacral spinal cord reduces bladder hyperreflexia in spinal cord injured rats. *J Urol* 168: 2269–2274. PMID: [12394773](https://pubmed.ncbi.nlm.nih.gov/12394773/)
30. Kruse MN, Belton AL, de Groat WC (1993) Changes in bladder and external urethral sphincter function after spinal cord injury in the rat. *Am J Physiol* 264: R1157–1163. PMID: [8322969](https://pubmed.ncbi.nlm.nih.gov/8322969/)
31. Jin Y, Neuhuber B, Singh A, Bouyer J, Lepore A, et al. (2011) Transplantation of human glial restricted progenitors and derived astrocytes into a contusion model of spinal cord injury. *J Neurotrauma* 28: 579–594. doi: [10.1089/neu.2010.1626](https://doi.org/10.1089/neu.2010.1626) PMID: [21222572](https://pubmed.ncbi.nlm.nih.gov/21222572/)
32. Mitsui T, Neuhuber B, Fischer I (2011) Acute administration of AMPA/Kainate blocker combined with delayed transplantation of neural precursors improves lower urinary tract function in spinal injured rats. *Brain Res* 1418: 23–31. doi: [10.1016/j.brainres.2011.08.023](https://doi.org/10.1016/j.brainres.2011.08.023) PMID: [21937028](https://pubmed.ncbi.nlm.nih.gov/21937028/)
33. Marson L (1995) Central nervous system neurons identified after injection of pseudorabies virus into the rat clitoris. *Neurosci Lett* 190: 41–44. PMID: [7624051](https://pubmed.ncbi.nlm.nih.gov/7624051/)
34. Lee JW, Erskine MS (2000) Pseudorabies virus tracing of neural pathways between the uterine cervix and CNS: effects of survival time, estrogen treatment, rhizotomy, and pelvic nerve transection. *J Comp Neurol* 418: 484–503. PMID: [10713575](https://pubmed.ncbi.nlm.nih.gov/10713575/)
35. Lepore AC, Bakshi A, Swanger SA, Rao MS, Fischer I (2005) Neural precursor cells can be delivered into the injured cervical spinal cord by intrathecal injection at the lumbar cord. *Brain Res* 1045: 206–216. PMID: [15910779](https://pubmed.ncbi.nlm.nih.gov/15910779/)
36. Gage FH (2000) Mammalian neural stem cells. *Science* 287: 1433–1438. PMID: [10688783](https://pubmed.ncbi.nlm.nih.gov/10688783/)

37. Gage FH, Coates PW, Palmer TD, Kuhn HG, Fisher LJ, et al. (1995) Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. *Proceedings of the National Academy of Sciences of the United States of America* 92: 11879–11883. PMID: [8524867](#)
38. Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 255: 1707–1710. PMID: [1553558](#)
39. Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Morshead CM, Fehlings MG (2006) Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. *Journal of Neuroscience* 26: 3377–3389. PMID: [16571744](#)
40. Ronaghi M, Erceg S, Moreno-Manzano V, Stojkovic M (2010) Challenges of stem cell therapy for spinal cord injury: human embryonic stem cells, endogenous neural stem cells, or induced pluripotent stem cells? *Stem Cells* 28: 93–99. doi: [10.1002/stem.253](#) PMID: [19904738](#)
41. Vaquero J, Zurita M (2009) Bone marrow stromal cells for spinal cord repair: a challenge for contemporary neurobiology. *Histol Histopathol* 24: 107–116. PMID: [19012250](#)
42. Hofstetter CP, Schwarz EJ, Hess D, Widenfalk J, El Manira A, et al. (2002) Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. *Proc Natl Acad Sci U S A* 99: 2199–2204. PMID: [11854516](#)
43. Erdogan B, Yacyoglu O, Feride Sahin I, Kayaselcuk F, Cemil B, et al. (2013) The effects of fetal allogeneic umbilical cord tissue transplant following experimental spinal cord injury on urinary bladder morphology. *Neurologia i Neurochirurgia Polska* 47: 138–144. PMID: [23650002](#)
44. Neuhuber B, Barshinger AL, Paul C, Shumsky JS, Mitsui T, et al. (2008) Stem cell delivery by lumbar puncture as a therapeutic alternative to direct injection into injured spinal cord. *J Neurosurg Spine* 9: 390–399. doi: [10.3171/SPI.2008.9.10.390](#) PMID: [18939929](#)