

# Urodynamic Parameters in Spinal Cord Injury-Induced Neurogenic Bladder Rats after Stem Cell Transplantation: A Narrative Review

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## What's Known

- Animal models of spinal cord injury, especially rats, are the most commonly used method to evaluate the efficacy of cell therapy in neurogenic bladder improvement and the most common assessment method is urodynamic studies.
- There are variations in the range of urodynamic parameters among the published studies.

## What's New

- Contusion injury is the most common spinal cord injury model. The average cell dose is about 1 million cells, transplanted 9 days after injury.
- In some urodynamic test results, to some extent, improvement in the function of the lower urinary system was observed. However, this improvement is far from full functional recovery.

## Abstract

Neurogenic bladder (NGB) secondary to spinal cord injury (SCI) is accompanied with several complications such as urinary tract deterioration, urinary incontinence, and consequently lower quality of life (QoL), significant morbidities, and occasionally death. Current therapeutic methods have some side effects and there is no treatment for the upper urinary tract injuries. Stem cell therapy is a promising method for treating this condition. However, the best timing and the best route of its transplantation have not yet been determined. Animal models of SCI, especially in rats, are the most commonly used method for evaluating the efficacy of cell therapy in NGB improvement, and the most common assessment method is the urodynamic studies (UDS). However, there are variations in the range of UDS parameters among the published studies. The current review aimed to discuss the effect of stem cell transplantation on bladder dysfunction recovery based on urodynamic parameters after SCI in rats. For this purpose, the cell source, doses, the route of administration, and the complete UDS equipment and its parameters were summarized in SCI models in rats. In some urodynamic test results, to some extent, an improvement in the lower urinary system function was observed in each treatment group. However, this improvement was far from full functional recovery. The average cell dose was about 1 million cells in every injected site. In most studies, the stem cells (SCs) were transplanted 9 days after the injury using PE-50 and PE-60. Many researchers have recommended further experimental and clinical studies to confirm this treatment modality.

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## Introduction

Neurogenic bladder (NGB) is a common condition in most neurological diseases such as spinal cord injury (SCI),<sup>1</sup> Parkinson's disease,<sup>2</sup> multiple sclerosis,<sup>3</sup> and cerebral vascular accident/stroke,<sup>4</sup> which has a negative impact on quality of life (QoL).<sup>5</sup> The International Continence Society (ICS) has defined overactive bladder (OAB) symptoms as urgency (with or without urge incontinence), daytime frequency, and nocturia. In the presence of a neurological condition, this problem is called

neurogenic OAB.<sup>6</sup> Urine stasis and upper urinary tract injury following NGB arises from high detrusor pressure during the filling phase (poor compliance) and additional contraction of the detrusor against the closed sphincter (detrusor sphincter dyssynergia).<sup>7, 8</sup> The prevalence of NGB in patients with SCI is 70% to 84%.<sup>9</sup> NGB secondary to SCI is accompanied with several complications such as urinary tract deterioration,<sup>10, 11</sup> urinary incontinence<sup>12</sup> and consequently lower QoL,<sup>13</sup> significant morbidities and occasionally death.<sup>14</sup> Numerous therapies for NGB including physical and psychological methods,<sup>15</sup> electrical stimulation,<sup>16</sup> medication,<sup>17</sup> and surgery<sup>18</sup> have been developed. However, these methods have some side effects and sometimes resulted in incomplete recovery.<sup>8</sup>

Stem cell transplantation and tissue engineering are the two important options that may overcome the limitations of the current therapies.<sup>19-21</sup> Cell therapy is a sub-type of regenerative medicine. In this process, SCs are introduced into the tissue to treat a disease with or without gene therapy.<sup>22</sup> The main SCs used for cell therapy are multipotent mesenchymal stem cells (MSCs)<sup>23, 24</sup> with two important abilities, namely self-renewing and differentiation into various cell types.<sup>25</sup> MSCs are derived from bone marrow (BM-MSCs),<sup>26</sup> skeletal muscle (Sk-MSCs),<sup>27</sup> umbilical cord (UC-MSCs),<sup>28</sup> and adipose tissue.<sup>29, 30</sup> Another source is adult tissue such as oral mucosa.<sup>31</sup> For bladder regeneration, MSCs showed better results in comparison with using differentiated SCs.<sup>32</sup> While numerous reports have shown the potential of these cells to replace or transplant in the lungs, liver, heart, and brain, data on improved bladder function is scarce.<sup>33-35</sup> Some researches focused on the role of neural progenitor SCs, as multipotent adult SCs, which exist in the central nervous system, in the regeneration of animal models.<sup>36</sup> The self-renewal potency and formation of new neurons and glial cells led researchers to use these cells.<sup>37</sup>

Bladder substitutes, using synthetic or natural materials, have been developed in recent years. However, despite numerous attempts, it is still an immature process because of mechanical, structural, functional, or biocompatibility problems. Bladder acellular matrix (BAM)-based scaffold obtained from donor's bladders may overcome these problems. In this process, the cell and its components are removed from the bladder and then the tissue matrix with collagen, elastin, fibronectin, glycosaminoglycans, proteoglycans, and growth factors contents are placed.<sup>38</sup>

The current review aimed to discuss the effect of stem cell transplantation on bladder dysfunction

recovery based on urodynamic parameters after SCI in rats. For this purpose, the cell source, doses, the route of administration, and the complete UDS equipment and its parameters were summarized in SCI models in rats.

### *Diagnosis of NGB*

UDS is an important factor in the diagnosis of urethral and bladder dysfunction.<sup>39</sup> According to the definition of ICS, UDS is the study of lower urinary tract physiology and the urinary hydrodynamic transmission.<sup>6</sup> Rodents, most commonly rats, are widely used to show urinary storage and voiding function both in normal conditions and in disease models<sup>40</sup> using cystometry as a common methodology. Cystometry in rodents gives important information about the physiology and pharmacology of the bladder.<sup>41</sup>

### *Cystometry in Rodents*

There are morphological and functional differences between the bladder of humans and rats.<sup>41</sup> In rats, bladder contraction is mediated by ATP, whereas in humans it is contracted by the mediation of acetylcholine.<sup>40-42</sup> Cystometric parameters in rodents are poorly defined and the used criteria are different from humans. Therefore, it is important to define the terminology on what is measured and avoid using the terminology for human cystometry.<sup>41</sup>

The reviewed articles had considered two different methods for urodynamic testing in rats. The first method was performed by using a cystometry catheter (PE-50) "Y" connected to a continuous infusion system and to a polygraph. In this method, vesical pressure during the filling and voiding phase is determined and the maximum urethral closure pressure is calculated with an indirect method.<sup>40</sup> The second method is performed using a catheter (external diameter of 0.64 mm and internal diameter of 0.5 mm) with two orifices inserted via the urethra to the bladder and "Y" connected to the infusion pump and polygraph. In this method, the behavior of the bladder during urination is determined.<sup>43</sup>

Anderson reported on a rodent UDS model<sup>40</sup> and noted that a control or sham group should be present in every cystometric study.<sup>40</sup> The common parameters that are usually reported in UDS include pressure parameters such as the baseline pressure or minimum pressure between two micturitions (usually between 10 to 20 cmH<sub>2</sub>O), intermicturition pressure or mean pressure between two micturitions (related to the occurrence of non-voiding contractions), threshold pressure or intravesical pressure (immediately before micturition, maximum

pressure during a micturition cycle or peak pressure, maximum voiding pressure, or maximum intravesical pressure), micturition frequency (the number of micturitions per hour), bladder capacity (determined as infused volume divided by the number of micturitions, provided there is no residual volume), and ICI (defined as the time period between two maximum voiding pressures). Volume parameters are the other cystometric parameters in the evaluation of the rodent bladder. It includes micturition volume (volume after a micturition minus volume before a micturition) and residual volume (the volume remaining in the bladder after voiding). Another important parameter of rodent UDS is bladder compliance (bladder capacity divided by threshold minus basal pressure).<sup>40</sup>

#### *Models of SCI to Induce NGB*

The contusion model of SCI is reported in some articles at the T10-T12 vertebral level,<sup>44</sup> T8-T9 level of the spinal cord,<sup>23</sup> at mid-thoracic level of T9,<sup>45</sup> at the 10<sup>th</sup> thoracic level of the spinal cord segment,<sup>46</sup> with the NYU impactor (10 g, 25 mm)<sup>47</sup> injury at the T9 vertebral level of the spinal cord<sup>48</sup> or laminectomy of the T10 spinal vertebrae, and complete transection at the lower thoracic level of the spinal cord.<sup>49</sup> Temeltas and others performed a laminectomy, following anesthesia, at the T9-T10 level and induced a hemisection SCI model via dura incision above the dorsal root entry zone and cutting with micro-scissors at the rostral and caudal extents of the injury. Then, aspiration was done to ablate only the lateral white matter tracts and a minimal portion of the dorsal and ventral gray matter.<sup>50</sup>

The contusion injury model is the most common SCI model. In this model, the dorsal region of the spinal cord is damaged or destroyed. Rostral to the lumbosacral level of SCI leads to bladder hyperreflexia, detrusor sphincter dyssynergia, and finally bladder hypertrophy.<sup>49</sup> Complete deterioration of bladder compliance, function, infection, and lower urinary tract complications are the other sequels of SCI.<sup>51</sup> In these studies, the mechanism of bladder dysfunction induced by SCI reported overactivity.

#### *The Timing of SCs Transplantation After Injury*

Different studies used different timings for the transplantation of SCs. It ranged from the immediate transplantation of cells after SCI<sup>44</sup> to 8 weeks after SCI.<sup>49</sup> However, in most studies, the SCs were transplanted nine days after injury. Cho and others<sup>44</sup> infused 100  $\mu$ l of oral mucosa SCs immediately after SCI. Park and others<sup>23</sup> used the timing of nine days after injury in which

human MSCs were placed in the contusion site of the spinal cord. Sandner<sup>45</sup> injected bone marrow stromal cells three days after inducing SCI in the center of the lesion. Obara<sup>46</sup> grafted BAM 2-3 weeks after SCI in rats that had undergone partial cystectomy 13 weeks after contusion. Jin<sup>47</sup> transplanted neural progenitor cells by using a 10- $\mu$ l NanoFil™ syringe (World Precision Instruments, USA) with a 33-gauge needle. In their process, the dura in the center of the lesion was not opened and remained for one minute after the injection.

In a study conducted by Lee,<sup>48</sup> human bone marrow MSCs labeled with magnetic nanoparticles were transplanted into six areas of the bladder muscle of rats four weeks after the SCI operation. Urakami<sup>49</sup> performed bladder replacement surgery eight weeks post-injury. The urinary bladder was exposed via a small suprapubic incision and partial cystectomy (50%), then BAM graft was anastomosed to the host bladder with running and interlocking 7-0 absorbable sutures. Temeltas and others<sup>50</sup> implanted a mixed population of neuronal restricted precursors (NRP)/glial restricted precursors (GRP) or culture medium transplantation, into the injury cavity nine days after hemisection.

The best route of administration and site of transplantation for different diseases and the possible contraindication of clinical usage are still unknown.<sup>32</sup> Moreover, the time of cell transplantation is very important<sup>52</sup> for the therapeutic effect.<sup>32</sup> The characteristics of the reviewed studies are described in table 1.

#### *The Methods and Time of Catheterization to Perform Cystometry after SCI*

In a study by Cho and others,<sup>44</sup> after anesthesia, a sterile polyethylene catheter (PE50) was inserted into the bladder connected to a pressure transducer (Harvard Apparatus Inc., USA) to record the intravesical pressure as well as a syringe pump to infuse saline into the bladder. After emptying the bladder, cystometry was performed. Park and others<sup>23</sup> conducted cystometry at 28 days and 56 days after transplantation. Sandner<sup>45</sup> catheterized the bladder 3-4 days prior to urodynamic measurements. After anesthesia, the bladder was exposed by a midline incision in the low abdominal wall and a PE catheter with a cuff was inserted into the bladder dome and secured by a purse-string suture and externalized through the skin in the back of the neck. The external tip of the catheter was closed thermally, to prevent leakage and infection, and sutured to the skin until the urodynamic measurements. Urodynamic measurements were performed 8.5

**Table 1:** Characteristic of SCI and stem cell types in the reviewed studies

Reference	SCI type	Device name for injury	The number of rodents in each group	Cell type for transplantation	Cell labeling	Time of transplantation after SCI	Route of transplantation	Cell count	Injured vertebrate segment
<b>Cho<sup>44</sup></b>	Needle-stick injury	22-gauge needle	10	Oral mucosa stem cells		Just after SCI	Stem cells were infused over the course of 1 min by using a 22-gauge insert vein (IV) catheter intrathecal	100 µl	T11
<b>Park<sup>23</sup></b>	Contusion	Multicenter animal SCI study (MASIS) impactor (Rutgers, The State University of New Jersey, Newark, NJ), a 10-gram rod was dropped from a vertical distance of 25 mm	6-11	hMSCs		9 days after injury	Into the injured spinal cord via Hamilton syringe	5 µL	T9
<b>Obara<sup>46</sup></b>	Compression	Compression with a 40-gram rod (tip area: 3.0-2.2 mm) placed on the exposed dura for 30 minutes	5 and 15	BAM grafting		2 to 3 weeks after BAM grafting	A BAM was firmly anastomosed to the host bladder with running and interlocking 7-0 absorbable sutures.		T10
<b>Sander<sup>45</sup></b>	Contusion	Infinite Horizon (IH) impactor SCI device (Precision Systems and Instrumentation, Lexington, KY, USA) with an impact force of 200 kilodynes (kdyn)	6-8	BMSCs		3 days postinjury	Into the center of the lesion at a maximum depth of 1.5 mm using a pulled glass pipette (100 µm internal diameter) and a Picospritzer II	1 × 10 <sup>5</sup>	T9
<b>Urakami<sup>49</sup></b>	Transection	Micro scissors		BAM grafting		8 weeks after SCI (bladder replacement)	The BAMG was anastomosed to the host bladder with running and interlocking 7-0 absorbable sutures		T10
<b>Temeltas<sup>50</sup></b>	Hemisection	Micro scissors	6-10	NRP/GRP		9 days after hemisection	Into the injury cavity	1 × 10 <sup>6</sup>	T9-T10
<b>JIN<sup>47</sup></b>	Contusion	NYU impactor (10 g, 25 mm)	9-10	NPCs		13 weeks after SCI	Using a 10-µl NanoFil syringe with a 33-gauge needle, 1 × 10 <sup>5</sup> cell/µl were injected into the lesion center along the midline (4 µl) and rostral and caudal to the lesion along the midline (3 µl/each).		T10
<b>Lee<sup>48</sup></b>	Contusion	Chung-Ang University Hospital Model 2.0 (CAUH-2) pneumatic impactor (3 mm depth)	10	BM3.B10	Fluorescent MNPs	4 weeks after SCI	Into the bladder muscle of the rats in six areas	1 × 10 <sup>6</sup>	T9

BAM: Bladder acellular matrix; hMSCs: Human mesenchymal stem cells; BMSCs: Bone marrow stromal cells; NRP: Neuronal restricted precursors; GRP: Glial restricted precursors

weeks post-injury in conscious rats.

In a study by Obara,<sup>46</sup> cystometry was performed at 5, 7, and 15 weeks in the intact-BAM and SCI-BAM rats. A polyethylene catheter was placed via the urethra into the bladder and was connected to the pressure transducers and then the pressure signals were recorded. Jin<sup>47</sup> performed cystometry in conscious rats eight weeks after transplantation (22 weeks after initial injury) by using a polyethylene catheter. After catheterization, the rats recovered for 1-2 hours and were then placed in a restraining cage (KN-326, Japan). A pressure transducer (Bladder pressure transducer, Germany) and infusion pump (STC-523, Japan) were used in this procedure. In a study by Lee,<sup>48</sup> voiding response was assessed at four weeks after transplantation. After anesthesia, the bladder was exposed via a midline abdominal incision and a catheter was inserted through a small incision in the bladder dome. After passing the other end of the catheter subcutaneously, its end was exited through the skin. Urakami<sup>49</sup> performed cystometry before grafting and repeated eight weeks after bladder replacement. After anesthesia, cystometry was performed after emptying the bladder. In a study by Temeltas and others,<sup>50</sup> UDS was conducted four weeks after transplantation similar to the previously mentioned method.

The time of catheterization to perform UDS varied among different studies. However, the timing was in the range of 2 to 8 weeks after transplantation. The catheter was inserted via the urethra or the bladder, usually in the dome, secured by a purse-string suture, tunneled subcutaneously, and externalized through the skin at the back of the neck. In these studies, PE-50 and PE-60 were used. However, in other UDS in rats, PE-10,<sup>53</sup> PE-50,<sup>54-57</sup> PE-60,<sup>58</sup> PE-90,<sup>59, 60</sup> and PE-100<sup>61</sup> were used.

#### *The Rate of Infusion into the Bladder to Induce Micturition to Perform Cystometry and Measure Parameters*

Cho and others<sup>44</sup> infused 0.5 ml normal saline and conducted cystometry. Park and others<sup>23</sup> infused 10 ml/hour normal saline at room temperature into the bladder. Then, the rate was reduced to 5 ml/hour. For stabilizing the micturition cycles, after the first void, the infusion was stopped every 30 minutes. Detrusor pressure, timing, and frequency of voiding were recorded. It should be noted that the cystometry in their study was conducted under the anesthetic vapor with low level (0.5%) to reduce the effect of anesthesia on the micturition cycle.

In a study by Sander,<sup>45</sup> to measure repeated

micturition cycles, the bladder was filled via the implanted catheter with saline for 30-45 minutes at a rate of 6 ml/h. During UDS, the bladder pressure and urine volume were recorded. To measure micturition volume, released fluid was collected through a funnel into a cup connected to a force transducer. For pressure measurements, the bladder catheter was connected via a three-way stopcock to a pressure transducer and to a syringe pump. Both transducers were connected to an amplifier and a data acquisition system. The measured parameters were micturition volume (MV), micturition frequency (MF per hour), basal pressure ( $P_{base}$ : minimum pressure between two micturition events), maximum voiding pressure ( $P_{max}$ : maximum bladder pressure during a micturition cycle), and pressure difference ( $P_{diff}$ :  $P_{max} - P_{base}$ ).

In a study by Obara,<sup>46</sup> saline 0.9% at room temperature was infused into the bladder continuously at a constant rate of 0.1ml/min. They only checked the leak-point volume and pressure when saline leaked from the meatus around the tube in anesthetic rats. Jin<sup>47</sup> performed UDS by infusion saline with a rate of 0.1 ml/min. Lee<sup>45</sup> recorded UDS by infusion of physiological saline at room temperature into the bladder at a rate of 0.04 ml/min. Urakami<sup>49</sup> performed a cystometry with saline infusion into the bladder at the rate of 0.2 ml/min. The measured parameters were bladder pressure during the filling phase, bladder capacity, threshold voiding pressure, bladder compliance (by dividing the bladder capacity by the threshold voiding pressure), voided volume, post-void residual volume, uninhibited detrusor contractions (UIC), maximal amplitude of UIC, and infused saline volume at the first UIC (table 2).

Temeltas and others,<sup>50</sup> 4-6 hours after rat recovery from anesthesia, infused saline at room temperature at the rate of 0.2 ml per minute and monitored the intravesical pressure. The saline infusion was halted during the micturition process. The voiding saline from the rat's urethral meatus was measured to a designated voided volume and post-void residual urine volume was then measured. The residual saline amount was withdrawn via an intravesical catheter and the bladder of the rats was squeezed manually on the abdominal wall and the remaining intravesical amount was collected. Voided volume and post-void residual volume was calculated as the bladder capacity. Different infusion rates have been described for rat urodynamics from 2.4 ml/hr to 11 ml/hr.<sup>40, 53-62</sup> In these studies, the rate of saline infusion was in the range of 0.04 ml/min to 0.1 ml/min or 6 ml/h. The characteristics of urodynamics of the studies are described in table 2.

**Table 2:** Characteristic of UDS in the reviewed studies

Reference	The time of cystometry after bladder catheterization	Time of catheterization to perform cystometry after SCI	The type of catheter used for cystometry	Animals status in cystometry (conscious or anesthetized)	The type of anesthetic drug for catheterization	Cystometry set-up	The rate of infusion into bladder
Cho <sup>44</sup>	Just before cystometry	21 days after SCI	PE-50	Anesthetized	Zoletil	Pressure transducer and a syringe pump (Harvard Apparatus, Holliston, MA, USA) monitored using LabScribe (iWork System, Inc., Dover, NH, USA).	0.50 ml/min
Park <sup>23</sup>	Just before cystometry	28 and 56 days after injury	Double lumen polyethylene catheter (PE-160 and PE-50; Clay Adams, Parsippany, NJ, USA)	anesthetized	Isoflurane	Pressure transducer by polygraph (Grass polygraph model 7E, Quincy, MA, USA) and infusion pump (Baxter, Deerfield, IL, USA).	First 10 ml/hour and then 5 ml/hour
Obara <sup>46</sup>	Just before cystometry	5, 7, and 15 weeks after SCI	PE-60 (Clay Adams, Parsippany, NJ, USA)	Anesthetized	Pentobarbital sodium	Pressure transducers (Statham P-23, Gulton-Statham Transducers, CA, USA) and recorded by (Menuet Compact, Dantec Medical A/S, Denmark).	0.10 ml/min
Sander <sup>45</sup>	3 to 4 days prior to urodynamic measurements	8.5 weeks post injury	PE catheter (diameter 2.1 mm, PE-50 (Schubert Medizinprodukte, Wackersdorf, Germany)	Conscious	Ketamine, xylazine, and acepromazine	Force transducer (MLT1030/D wide range force transducer; AD Instruments, Oxford, UK), pressure transducer (MLT0699 disposable BP transducer; AD Instruments, New Zealand) and to a syringe pump (KR Analytical, Cheshire, UK), data acquisition system (PowerLab 8/35; AD Instruments, New Zealand)	6 ml/h
Urakami <sup>49</sup>	Just before cystometry	Before grafting, 8 and 16 weeks after SCI and bladder replacement	22-gauge catheter	Anesthetized	Ketamine		0.20 ml/min
Temeltas <sup>50</sup>	4 to 6 hours after recovery	4 weeks after transplantation	PE-50		ketamine+xylazine	Biopac Systems™ device	0.20 ml/min
JIN <sup>47</sup>	1-2 hour before urodynamic measurements	22 weeks after SCI and 8 weeks after transplantation	PE-60 (Clay Adams, Parsippany, NJ, USA)	Conscious	Isoflurane	Pressure transducer (BLPR; World Precision Instruments) (BLPR; Bladder pressure transducer, Germany) and infusion pump (STC-523; Terumo, Tokyo, Japan)	0.10 ml/min
Lee <sup>48</sup>	5-6 hour before urodynamic measurements	4 weeks after cell transplantation and 8 weeks after injury	PE-50 (Harvard Clinical Technology, Inc., South Natick, MA, USA)	Conscious	Isoflurane		0.04 ml/min

PE: Polyethylene tube

### The Results of UDS

Cho<sup>44</sup> determined the contraction pressure  $2.55 \pm 0.22$  cmH<sub>2</sub>O in the sham-operation group,  $6.49 \pm 0.77$  cmH<sub>2</sub>O in the SCI-induced group, and  $3.05 \pm 0.14$  cmH<sub>2</sub>O in the SCI-induced and oral mucosa stem cell transplantation group. The results showed that bladder contraction pressure and contraction time were increased in the SCI group, whereas they were improved after the transplantation of oral mucosa SCs.

Park and others<sup>23</sup> showed that the voiding frequency (time/min) was  $0.80 \pm 0.09$  and  $0.82 \pm 0.16$  in the two control groups and  $0.79 \pm 0.11$  in an experimental group at 28 days after injury. Sandner<sup>45</sup> observed a similar baseline bladder pressure, a slightly increased maximal voiding pressure, and a significantly increased pressure difference. In addition, spinal cord-injured animals frequently showed non-voiding contractions, which was not observed in naive animals. In treated animals, a tendency toward a decrease of the micturition frequency was reported compared to non-injected injured rats; although this difference was not statistically significant using ANOVA analysis ( $P=0.0535$  comparing all injured groups). The voided volume per micturition of all injured animals was decreased compared to intact animals, but was not significant using ANOVA analysis ( $P=0.06$ ). In both fibroblast-grafted and BMSC grafted animals compared to the injured control animals, the voided volume per micturition was slightly high and the difference among the injured group and control group was not statistically significant using ANOVA analysis ( $P=0.28$ ).

In a study by Obara,<sup>46</sup> due to the phenobarbital anesthesia effect on suppressing the neural circuit of the micturition reflex, the intravesical pressure rose gradually until voiding was initiated. The leak point volume of the SCI rats was significantly higher than that of the intact rats, and the leak-point pressure of the SCI rats was significantly lower than the intact rats.

Jin<sup>47</sup> observed bladder deficiency in all experimental groups in comparison with the intact group. As shown in table 3, these values increased in all experimental groups compared to the intact group ( $P<0.05$ ). Non-voiding contractions in the intact group were zero, while in other groups were  $7.4 \pm 3.1$ ,  $6.0 \pm 0.6$ ,  $7.0 \pm 1.2$  and  $7.8 \pm 1.5$ , respectively. The voided volume (ml) ranged between 0.3 in the intact group to 4.13 in the group of NPCs+lentivirus vectors expressing chondroitinase and neurotrophic factors (N/C/G group). The bladder contraction duration and amplitude of micturition were also high in three of the four experimental groups compared to the intact animals, except for the N/C/G group, which

was similar to normal values. After combined treatment, bladder function improvement was observed. The interaction intervals (seconds) were  $148.9 \pm 10.5$  in the intact group,  $214 \pm 40.9$  in medium (control) group,  $289.8 \pm 78.8$  in neural progenitor cells transplants (NPCs) group,  $258.2 \pm 38.4$  in NPC+lentivirus vector expressing chondroitinase group, and  $230.5 \pm 69.2$  in NPC+lentivirus vectors expressing chondroitinase and neurotrophic factors. Bladder contraction duration (seconds) was  $19.1 \pm 2.5$ ,  $28.6 \pm 3.9$ ,  $28.2 \pm 6.2$ ,  $31.8 \pm 4.6$ , and  $18.9 \pm 3.5$ , respectively. The amplitude of micturition (cmH<sub>2</sub>O) was  $35.0 \pm 6.3$ ,  $44.3 \pm 7.8$ ,  $42.3 \pm 6.3$ ,  $47.9 \pm 7.2$ , and  $34.7 \pm 2.5$ , respectively. Residual volume (ml) was zero in the intact group, 0.177 in medium group, 0.1 in NPC group, 0.05 in N/C, and 0.05 in N/C/G group.

Lee and others<sup>48</sup> showed that following SCI, the inter-contraction interval (ICI: the interval between voids or reflex bladder contractions) was low. However, after cell transplantation, the inter-contraction interval was increased. According to their figures, the ICI (seconds) was about 340 in normal rats, 370 in the sham group, 50 in the SCI group, and about 160 in the SCI+B10 cell group. The pressure threshold (PT) was similar between the groups. The amount of PT (cmH<sub>2</sub>O) in the control group was about 8, 9 in the sham group, 7 in the SCI group, and about 9 in the SCI+B10 cell group. After SCI, the amount of maximum voiding pressure was increased, but it decreased after B10 cell transplantation.

In a study by Urakami,<sup>49</sup> eight weeks after spinalization, 22 rats (71.0%) had hyperreflexic bladders with UIC and nine rats (29.0%) had underactive bladders with no UIC. The mean bladder capacity and RUV of SCI rats ( $4.2 \pm 2.3$ ,  $1.5 \pm 1.6$  ml, respectively) were remarkably higher than normal rats ( $0.5 \pm 0.1$ ,  $0.05 \pm 0.02$  ml, respectively). Similar urodynamic results were seen in SCI-control rats (with no bladder replacement) eight weeks and 16 weeks after spinalization. No uninhibited contraction was observed in all ten normal rats. The bladder capacity was 1.0 ml and the maximum amplitude of the voiding contractions was 31.0 cm/H<sub>2</sub>O.

A typical cystometrogram in spinalized rat showed many large uninhibited non-voiding contractions (more than 15 cm/H<sub>2</sub>O) during saline infusion. The size of the contractions increased with time. Bladder capacity was approximately 2.5 ml, threshold pressure was 33.5 cm/H<sub>2</sub>O, and bladder compliance was 0.075. No uninhibited contractions during saline infusion were seen in a typical cystometrogram of SCI-induced underactive-bladder (areflexia). The bladder capacity exceeded 4.0 ml and bladder compliance was very high.

Table 3: Measured urodynamic parameters in SCI-induced neurogenic bladder in the reviewed studies

Reference	Groups	Contraction pressure (cmH2O) (mean±SD)	Contraction time (seconds) (mean±SD)	Voiding pressure (mean±SD)	Voided volume (mean±SD)	Voiding frequency (time/min) (mean±SD)	Maximal pressure (mean±SD)	Voiding pressure (VP) (mean±SD)	Pressure threshold (PT) (mean±SD)	Bladder capacity (mean±SD)	Bladder compliance (mean±SD)	Post-void residual volume (mean±SD)	Baseline pressure (cmH2O) (mean±SD)
Cho <sup>44</sup>	Control	2.55±0.22	8.09±0.43										
	Negative control	6.49±0.77	15.29±1.65										
Paik <sup>23</sup>	Experimental	*3.05±0.14	*13.36±0.40										
	Control 1 PBS (n=7)			0.80±0.09	9.21±5.30					1345.25±1206.27 mm <sup>3</sup>			
Lee <sup>48</sup>	Control 2 hFb (n=11)			0.82±0.16	9.60±4.20					1263.75±838.05 mm <sup>3</sup>			
	Experimental hMSC (n=9)			0.76±0.32	9.70±4.11					1279.57±1310.69 mm <sup>3</sup>			
*Sander <sup>45</sup>	Control												
	Sham						39.24±2.64		8.18±0.77				
	SCI						43.77±1.50		8.50±0.54				
	SCI+B10						77.73±2.64		7.13±0.72				
Urakam <sup>49</sup>	SCI						57.35±4.15*		8.54±0.31*				
	BMSC-grafted Fibroblast-grafted						72.60±9.56						26.38±6.38
Temeltas <sup>50</sup>	Control									0.50±0.10			
	Before graft After BAM-grafted										0.37±0.07	1.38±0.20	
JIN <sup>47</sup>	Sham			24.90±4.03	0.56					0.57±0.13			2.15±0.54
	SCI+neural restricted precursor/glia restricted precursor			*43.40±5.71	1.02					*1.64±0.13			3.38±0.30
Urakam <sup>49</sup>	SCI+BM-SCs			*53.10±7.88	0.90					*1.70±0.10		*0.79±0.20	*5.16±1.03
	SCI control			61.80±9.41	0.27					1.89±0.18		1.61±0.23	8.78±0.55
JIN <sup>47</sup>	Intact				0.30					0.3±0.00		0.00	
	Medium (control)				3.63					3.8±0.90		0.17	
Urakam <sup>49</sup>	NPC transplant			*5.20	*5.20					*5.3±1.20		*0.10	
	NPC transplant with Chase/LV (N/C)			*4.05	*4.05					*4.1±0.80		*0.05	
Urakam <sup>49</sup>	NPC transplant with Chase/ LV+BDNF/NT-3/LV (N/C/G)				*4.13					*4.2±1.30		*0.05	

\*P<0.05: Improvement of bladder function after stem cell transplantation or grafting. \*\*There was no quantitative information about the cystometric parameters in the text and the result was written based on figure information. BM-SCs: Bone marrow stromal cells; NPCs: Neural progenitor cells; Chase/LV: Lentiviral vector expressing chondroitinase; Chase/LV+BDNF/NT-3/LV: Lentiviral vector expressing chondroitinase and growth factor treatments, B10 cell: BM3.B10 (B10); hMSCs: Human mesenchymal stem cell; PBS: Phosphate-buffered saline; hFbs: Human fibroblasts



Temeltas<sup>50</sup> reported that the mean±SD of baseline pressure (cmH<sub>2</sub>O) in the sham operation group was 2.15±0.54, in the SCI group that received NRP/GRP was 3.38±0.30, in the SCI group that received bone marrow stromal cell transplantation 5.16±0.55, and in the SCI control group 8.78±0.55. The mean±SD of maximum bladder capacity (ml) was 0.57±0.13, 1.64±0.13, 1.70±0.10, and 1.89±0.18, respectively. The frequency of uninhibited detrusor contractions per minute was in the range of 0.20±0.44 to 5.80±2.58 and the amplitude (cmH<sub>2</sub>O) was in the range of 1.20±2.68 to 32.01±10.19. The mean±SD of voiding pressure (cmH<sub>2</sub>O) was 24.90±4.03 in the sham operation group, 43.40±5.71 in group 2, 53.10±7.88 in group 3, and 61.80±9.41 in the SCI control group. The mean±SD of voided volume (ml) was 0.56±0.12 in group 1, 1.02±0.08 in group 2, 0.90±0.18 in group 3, and 0.27±0.05 in group 4. Post-void residual volume (ml) was in the range of 0.05±0.07 to 1.61±0.23. Urodynamic parameters were poor in all other groups compared to the sham operation group (P=0.0001). After treatment, the level of baseline pressure, maximum capacity, and amplitude of involuntary contraction during the filling phase were improved (P=0.0001, P=0.001, and P=0.001, respectively). The number of involuntary contractions was not significantly different between the treatment groups and group 4. Mean voiding pressure and post-void residual urine volume in the treatment groups were lower than the control group (P=0.0001 and P=0.001, respectively), while voiding volume was greater (P=0.001).

In the reviewed articles, several cystometric parameters such as cystometric pressure parameters (baseline, intermicturition, threshold, maximal, voiding and non-voiding pressure), micturition frequency, volume parameters (voided volume, post-void residual volume), and bladder compliance were measured. In some studies, only contraction pressure and time with a range of 2-6 cmH<sub>2</sub>O and 8-15 seconds, respectively, were measured.<sup>44</sup> In another study, only maximal pressure was measured.<sup>23</sup> Three studies reported bladder capacity<sup>45, 49, 50</sup> with a minimum range of 0.3 to a maximum range of 5.3 ml.<sup>44, 46, 47</sup> Some important results of UDS in the above-mentioned studies are summarized in table 3.

## Discussion

SCI-induced bladder dysfunction is one of the most debilitating functional deficits.<sup>63,64</sup> Among different treatment methods, stem cell transplantation is promising. In some urodynamic

test results, to some extent, improvement in the function of the lower urinary system in each treatment group was observed. However, this improvement was far from full functional recovery. The remaining unknowns for the best therapeutic effect are the route of administration, cell doses, timing, the site of cell transplantation for different diseases, and possible contraindication of clinical usage.<sup>32, 46</sup> Another concern in the field of stem cell therapy is the diminishing regenerative potential of aged SCs. Thus, the success of future clinical trials will depend on experimental investigations.<sup>65</sup> There were different timings for stem cell transplantation among the reviewed studies, ranging from immediate transplantation of cells after SCI<sup>44</sup> to several weeks after SCI.<sup>49</sup> The average cell dose was about one million cells in every injected site and, in most studies, the SCs were transplanted nine days after injury. Transplanted cell survival is still another significant challenge that limits further recovery in SCI. In this regard, the main limitation is the high post-transplantation cell mortality.<sup>66</sup> Various strategies for improving the survival of SCs have been proposed.<sup>67</sup> However, none of the reviewed studies had mentioned such strategies.

To examine voiding functions, an implanted catheter into the bladder dome (by avoiding bladder outlet obstruction and providing the ability to perform urodynamic measurements in conscious rats) was preferred. Different infusion rates from 2.4 ml/hr to 11 ml/hr have been described for rat urodynamics. In the reviewed studies, the rate of saline infusion ranged from 0.04 ml/min to 0.1 ml/min or 6 ml/hr. PE-50 and PE-60 were also used; however, PE-10,<sup>53</sup> PE-50,<sup>54-57</sup> PE-60,<sup>58</sup> PE-90,<sup>59, 60</sup> and PE-100<sup>61</sup> were used in other urodynamic studies in rats. It was assumed that the thinner tubes (PE 10) had a higher resistance.<sup>68</sup>

Several cystometric parameters such as pressure parameters, volume parameters, and bladder compliance were measured. Some studies only measured the contraction pressure and time with a range of 2 to 6 cmH<sub>2</sub>O and 8 to 15 seconds, respectively. In another study, only the maximal pressure was measured. Three studies reported bladder capacity with a minimum range of 0.3 to a maximum range of 5.3 ml. Moreover, conscious rats had different bladder behavior compared to anesthetized rats. In the present review, approximately 50% of the studies were conducted on conscious rats and some others used common drugs such as isoflurane and/or ketamine for anesthesia. Ozkurkcugil surveyed the effect of different anesthetic drugs on cystometric parameters in comparison with the conscious condition.

The effect of ether and midazolam on the parameters were similar in both conditions. Propofol altered these parameters but was not statistically significant.<sup>69</sup>

Despite the claim that ketamine has no effect on cystometric parameters, Ozkurkucugil reported that it had a high depressant effect on micturition. Therefore, it may be not suitable for experimental studies using urodynamic parameters in rats.<sup>69</sup> Isoflurane may be the best choice for small surgical procedures, because it has a short half-life. However, in a study by Chang and others, isoflurane led to the prolongation of bladder inter-contractile intervals.<sup>70</sup> Hence, anesthetic properties should be taken into account in the experimental design and interpretation of urodynamic findings in rodent models.

### Conclusion

In the present study, the effect of stem cell transplantation on bladder dysfunction recovery based on urodynamic parameters after SCI in rats was reviewed. For this purpose, the cell source, doses, route of administration, and the complete UDS equipment and its parameters were summarized in SCI models in rodents. Among the reviewed studies, the contusion injury model was the most common SCI model. The average cell dose was about one million cells in every injected site. Additionally, the SCs were transplanted into the lesion cavity nine days after injury.

In some urodynamic test results, to some extent, an improvement in the function of the lower urinary system in each treatment group was observed. However, this improvement was far from full functional recovery. Since we did not assess its effect on the other neurological conditions, it cannot be generalized to other neurological diseases. Hence, it is recommended to conduct a comprehensive review of all neurological conditions that lead to NGB and to compare urodynamic parameters between treated and control groups.

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**Conflict of Interest:** None declared

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