World J Urol (2014) 32:1241–1248
DOI 10.1007/s00345-013-1200-6

ORIGINAL ARTICLE

Adipose-derived stem cells (ADSCs) and muscle precursor cells (MPCs) for the treatment of bladder voiding dysfunction

Mathias Tremp · Souzan Salemi · Remo Largo · Karl-Erik Andersson · Jan A. Plock · Tamer Aboushwareb · Tullio Sulser · Daniel Eberli

Received: 31 July 2013 / Accepted: 21 October 2013 / Published online: 12 November 2013

Abstract
Purpose Bladder outflow obstruction (BOO) is common in the elderly and can result in bladder voiding dysfunction (BVD) due to severe bladder muscle damage. The goal of this research was to evaluate the use of adult stem cells for the treatment of BVD due to decreased muscle contractility in a rat model.
Materials and methods Adipose-derived stem cells (ADSCs) and muscle precursor cells (MPCs) were harvested from male Lewis rats and expanded in culture. BOO was induced by tying a suture around the urethra. Six weeks after obstruction, the development of BVD was confirmed by cystometric analysis in conscious rats, histology and molecular investigations. Injection of ADSCs or MPCs into the bladder wall and synchronous deligation was performed 6 weeks after the obstruction. After stem-cell treatment, morphological and functional changes were assessed. Age-matched rats and animals without cellular therapy but deligation-only served as controls.
Results Voiding pressures decreased progressively 6 weeks after obstruction with increased bladder capacities. Structural changes of the detrusor muscle occurred during the time of obstruction with an increased connective tissue-to-smooth muscle ratio and decreased SMA/smoothelin expression. After stem-cell injection, improved voiding pressures and voiding volumes were observed together with recovered tissue architecture. RT-PCR and Western blotting showed an up-regulation of important contractile proteins.
Conclusions We established a reliable model for BVD and demonstrated that ADSCs and MPCs can prevent pathophysiological remodelling and provide regenerated bladder tissue and function.

Keywords Stem cell · Regenerative medicine · Urinary bladder · Autologous transplantation · Muscle contraction

Mathias Tremp and Souzan Salemi have contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00345-013-1200-6) contains supplementary material, which is available to authorized users.

M. Tremp · S. Salemi · R. Largo · T. Sulser · D. Eberli (✉)
Division of Urology, University Hospital Zürich (USZ), 8091 Zurich, Switzerland
e-mail: daniel.eberli@usz.ch

K.-E. Andersson · T. Aboushwareb
Wake Forest University Health Sciences, Wake Forest Institute for Regenerative Medicine, Medical Center Boulevard, Winston Salem, NC 27154-1094, USA

J. A. Plock
Division of Plastic and Reconstructive Surgery, Department of Surgery, University Hospital Zürich, 8091 Zurich, Switzerland

Introduction

Bladder outlet obstruction (BOO), such as benign prostatic hyperplasia, is a common disorder affecting 50–80 % of males over the age of 50 [1]. It causes various urinary symptoms and leads to frequency, urgency and urgency incontinence due to involuntary detrusor contractions in the storage phase, and to hesitancy and weak flow in the voiding phase with consequent urinary retention [2]. When identified and treated early, the long-term sequel of BOO can be avoided. However, if detected in the decompensated stage, the voiding dysfunction often remains after surgical treatment due to the loss of bladder smooth muscle [3].

Currently, no treatment modality reverses the underlying cause, which is the decreased bladder contractility. In most cases, a long-term indwelling catheter is necessary, entailing all its disadvantages.

Tissue engineering and cellular therapy offer novel strategies to potentially revert the deterioration of muscle tissue and improve bladder contractility. Most current strategies for tissue engineering depend upon a sample of autologous cells from the diseased organ of the patient. However, biopsies from patients with extensive end-stage organ failure and fibrosis of the bladder may not yield enough normal cells. Therefore, we have used easily accessible adult cells, namely adipose-derived stem cells (ADSCs) and muscle precursor cells (MPCs) as an alternative source in this study.

ADSCs are well-suited for regenerative medicine because of ease of harvest for autologous transplantation, high proliferation rates for ex vivo expansion and multilineage differentiation capacity [4]. We use the term MPCs for the pool of precursor cells harvested from muscle tissue expressing muscle-specific markers and giving rise to new muscle tissue [5]. MPCs have previously demonstrated long-term survival, the ability to form new myotubes [6], the formation of new motor units [7] and differentiation to smooth muscle cells [8]. The goal of this project is to evaluate the use of autologous stem cells for the treatment of bladder voiding dysfunction (BVD).

Materials and Methods

(Experimental procedures are in supplementary and available online).

Study design

For the generation of bladder hypocontractility, we used a modified model of BOO [9, 10]. Morphological, histological and functional data (cystometry) were collected 6 weeks after partial obstruction of the urethra. Cystometric analysis was performed without anaesthesia after implantation of a subcutaneously tunnelled catheter into the bladder. Healthy age-matched animals served as normal controls. All study animals had a partial urethral obstruction for 6 weeks followed by either deligation-only or deligation with cell injection (ADSCs or MPCs) (Figure S1).

Animal model generation

All animal research was performed in accordance with the Animal Ethics Committee and Animal Welfare Law. Borgal 24 % (Sulfadoxin+Trimethoprim) and Buprenorphin (Temgesic®, 2 ml Amp, 0.3 mg/ml, 0.1–0.2 mg/kg) were administered prior to surgery. Under anaesthesia with isoflurane 3 %, a 1 cm midline incision was made from the penoscrotal junction to the midscrotum to gain access to the bulbous urethra. The urethra was then isolated from the cavernous bodies. A sterile metal bar with a diameter of 0.91 mm was placed on the bulbous urethra and a 3-0 polypropylene suture was tied around both the urethra and the bar. The metal bar was then removed, leaving the prostatic urethra partially obstructed. A 3-0 vicryl suture was used for wound closure.

Results

We analysed data of 53 male Lewis rats (6–8 weeks old weighing 225–300 g). In group one, 12 rats were evaluated to confirm bladder hypocontractility. In group two, 12 animals received partial urethral obstruction and deligation 6 weeks later. In groups three and four, deligation and stem-cell injection (12 animals with ADSCs and 12 animals with MPCs, respectively) into the bladder wall was performed 6 weeks after the obstruction. Five healthy age-matched rats served as normal controls. Animals with signs of acute urinary retention or infection after obstruction were terminated early and replaced.

Bladder hypocontractility 6 weeks after partial urethral obstruction

Obstructed rats had a significantly larger bladder weight (178.3 ± 20.9 mg vs. 57.6 ± 5.2 mg, $p = 0.0011$) and a lower bladder wall thickness (434.7 ± 39.0 µm vs. 815.8 ± 177 µm, $p = 0.0067$) than normal controls (Fig. 1). Histological examination showed an increased connective tissue-to-smooth muscle ratio in the obstructed bladder (0.6 ± 0.05 vs. 0.47 ± 0.12) compared with normal controls (Fig. 2). Functional analysis in the obstructed bladder showed significantly lower maximum peak pressure, threshold pressure as well as lower voiding volumes. Furthermore, mean values of average slope/cm^2 H_2O was significantly lower compared with the normal bladder (Figs. 1 and 4).

Cellular therapy with ADSCs and MPCs

ADSCs with an overall purity of more than 65 % for CD44 and CD29 and low levels of CD34 (<5 %) were used for this study. MPCs with a purity of more than 90 % for MYHC and 60 % for Desmin and Actinin were injected (Figure S2). After harvesting the bladder, there were no signs of tumour formation, adhesions or inflammation.

Data collected 4 and 8 weeks after deligation and stem-cell injection were likewise, therefore they were pooled

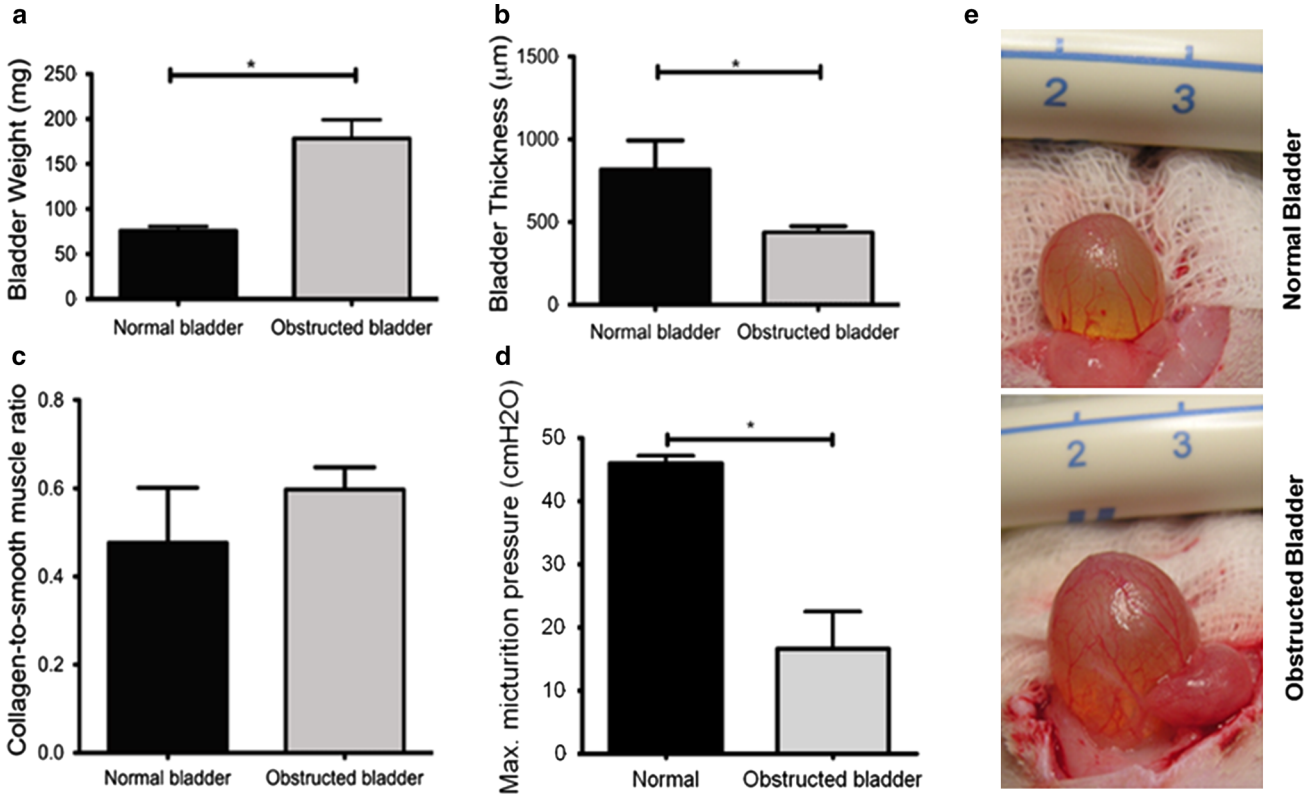


Fig. 1 Morphological and functional characterization of normal and obstructed bladder 6 weeks after partial urethral obstruction. Bladder weight (**a**), wall thickness (**b**), connective tissue-to-smooth muscle ratio (**c**) and micturition pressure (**d**). Representative images of a normal and obstructed bladder (**e**)

and labelled "post-treatment". Stem-cell therapy improved tissue architecture with increased smooth muscle clusters (Fig. 2). PKH-labelled cells were tracked after 4 weeks and found to be arranged in dense muscle clusters. Connective tissue-to-smooth muscle ratio was lower in the ADSCs group (0.36 ± 0.12) compared with the obstructed group (0.6 ± 0.05), whereas it remained high in the deligation group (0.67 ± 0.14). Similarly, the MPCs group revealed a lower connective tissue-to-smooth muscle ratio than the obstructed bladder (0.4 ± 0.06).

To assess the tissue of the bladder wall, immunostaining of smooth muscle-associated proteins were performed (Fig. 3). SMA was detected in muscle areas of all treated samples with a lower expression in the animals with deligation. These findings were confirmed by staining of smoothelin, the key contractile protein with high levels of expression in animals after stem-cell injection.

Functional assessment after cellular treatment

Cystometry studies demonstrated a higher maximum micturition and threshold pressure after cell injection with ADSCs and MPCs compared with the obstructed and the deligated groups (Figs. 4 and 5). The maximum peak pressure using ADSC (41.7 ± 8.4 cmH_2O, $n = 6$) and MPC ($42.5 \pm 0.1.8$ cm H_2O, $n = 5$) were both significantly ($p < 0.05$) higher than in the obstructed group (16.6 ± 5.8 cmH_2O, $n = 6$), showing improved function similar to untreated normal control animals (45.9 ± 1.24 cmH_2O, $n = 5$). Furthermore, an increased threshold pressure was found after ADSCs (13.0 ± 4.3 cmH_2O) and MPCs ($13.81 \pm 2.2cmH_2O$) injections, whereas in the deligation group (8.7 ± 2.9 cmH_2O, $n = 6$), a lower threshold pressure was noticed. A similar pattern of improved average slope was observed after ADSCs and MPCs treatments (0.7 ± 0.2 cmH_2O and 1.8 ± 0.19 cmH_2O, respectively) compared with the obstructed group (0.42 ± 0.3 cmH_2O). The ADSCs and MPCs groups (0.82 ± 0.2 and 0.95 ± 0.2 ml, respectively) showed an improved bladder voiding volume compared with group one (0.55 ± 0.19 ml). Conversely, the deligation group (0.5 ± 0.13 ml) did not reach the levels of the ADSCs- or MPCs-treated groups.

Gene expression and Western blot (WB)

At mRNA level, an increased expression of calponin, MYH11 and smoothelin was observed in the stem-cell- treated groups

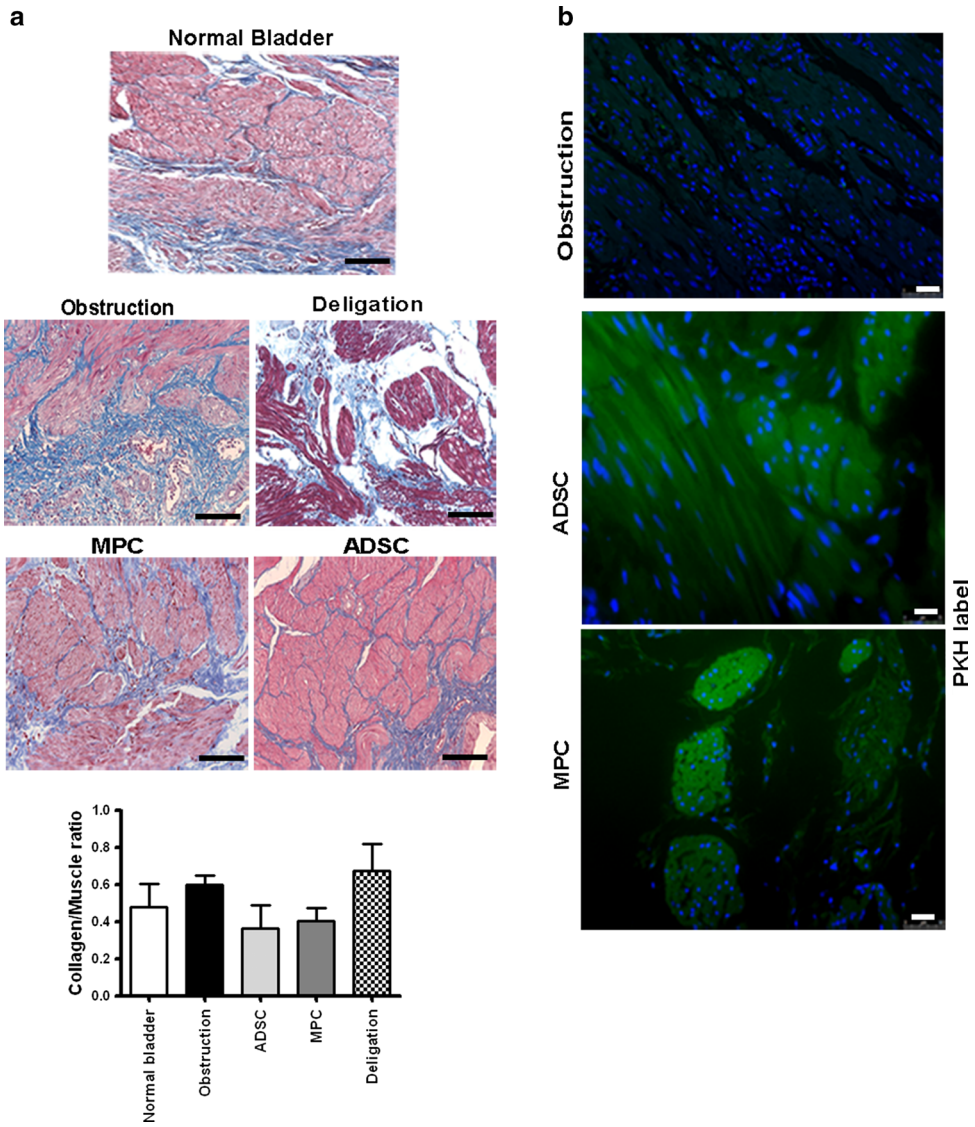


Fig. 2 **a** Masson's Trichrome staining 4 and 8 weeks after cell injection, with quantitative analysis of connective tissue-to-smooth muscle ratio. **b** Representative images of PKH 67-labelled (*green*) cells arranged in dense clusters after stem-cell injection

compared with obstructed animals (Figure S3). WB analysis revealed increased levels of smoothelin, calponin and MYH11 in the treated animals compared with the deligated animals. Above all, smoothelin was higher expressed in the stem-cell-treated groups than in the deligation groups, whereas only a weak basal expression of calponin and MYH11 was found in the deligation group.

Discussion

Cellular therapy using autologous cells improving bladder function would certainly represent a major medical breakthrough. The mechanism of bladder restoration can be explained by a combination of cell differentiation, paracrine factors from the stem cells and microenvironmental signals [11]. Homing and recruitment of other haematopoietic or mesenchymal stem cells to sites of injury chemoattracted through microenvironment niche conditions have been suggested to accelerate bladder regeneration [12]. Certain cues signal the stem cells to activate their differentiation potential for the regeneration or repopulation of a tissue [12] [13] [14]. In this study, we mimicked this process by injecting autologous stem cells directly into the damaged bladder tissue at multiple locations. Furthermore, we determined whether cell treatment could improve the bladder architecture in rats with BOO through smooth muscle restoration and regeneration. Thus, the present study provides insights into the possible use of two adult stem cell sources for bladder bioengineering.

In a recent study, autologous ADSCs were seeded onto bladder acellular matrix grafts for bladder

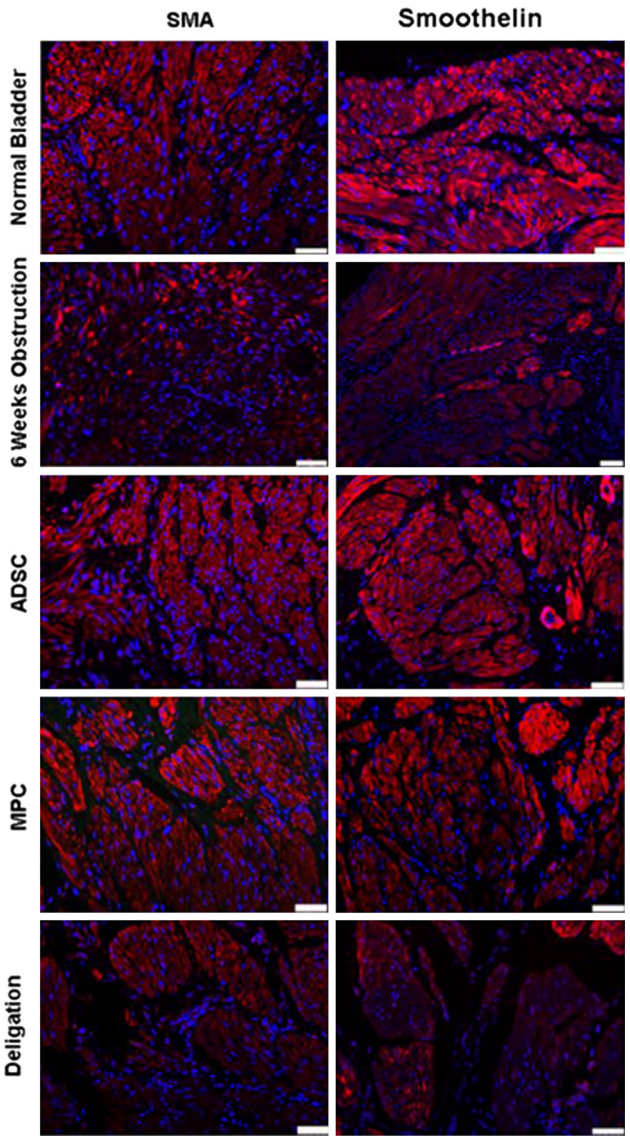


Fig. 3 Representative immunohistochemical staining of the different groups. Tissue sections were immunostained for smooth muscle markers SMA and smoothelin. Proteins were detected using CY3-conjugated secondary antibody (*red*) and DAPI (*blue*). *Red colour* indicates expression of each protein and *blue colour* indicates the nucleus. *Scale bar* = 100 μm

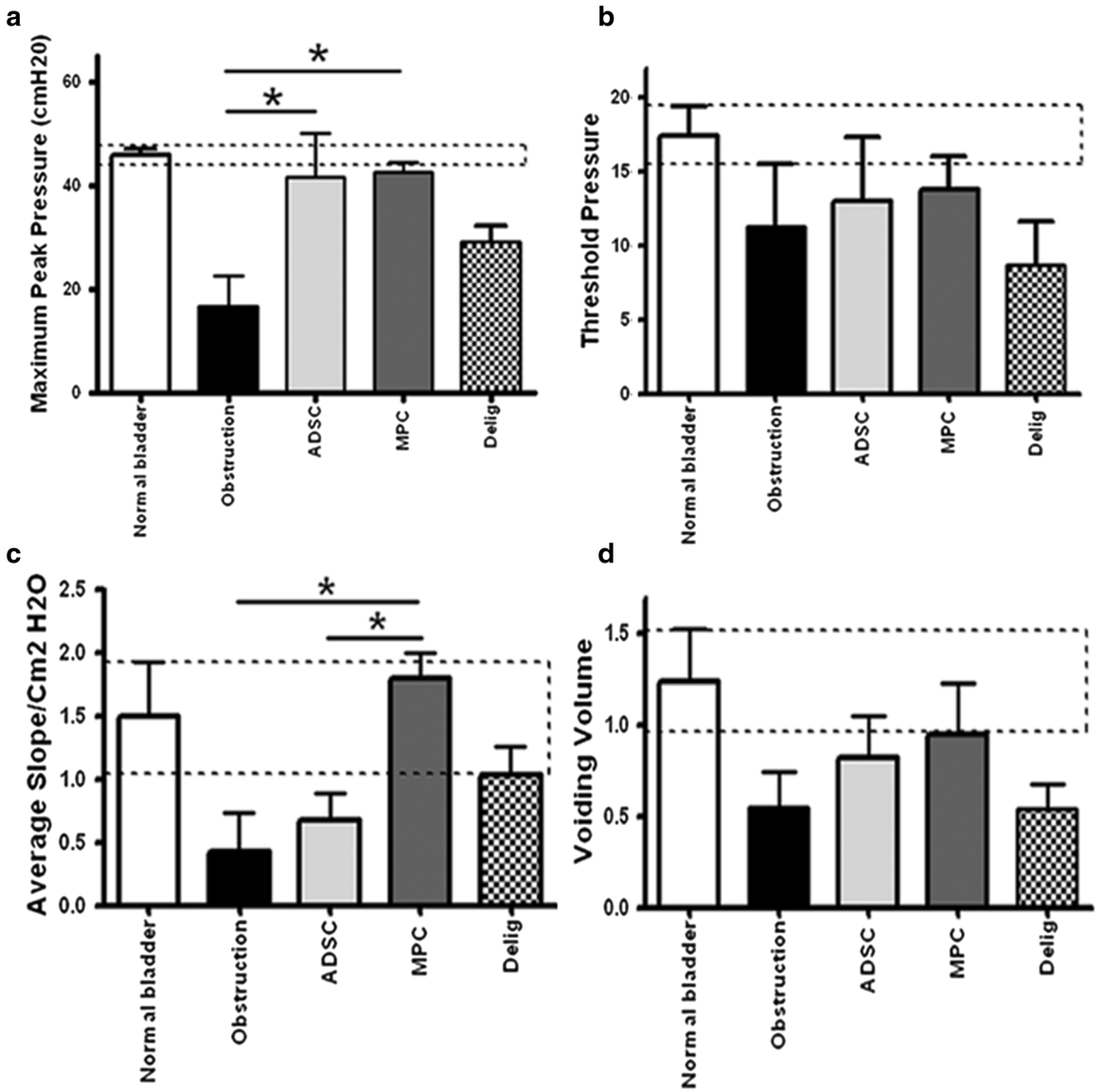
reconstruction in a rabbit model [15]. ADSCs promoted the regeneration of smooth muscle, neural tissue and also the compound graft [15]. In addition, ADSCs were found to improve urge [16] and stress urinary incontinence in a rat model [17]. MPCs that are predecessors of the satellite cells are considered not to be restricted to the myogenic or mesenchymal tissues [18]. In a recent study, muscle-derived multipotent stem cells (CD34+/45- and CD34-/CD45-) from green fluorescent protein transgenic mouse muscles were transplanted into a nude rat model for regeneration of neurogenic bladder dysfunction [19]. A significantly higher functional recovery 4 weeks after cell injection was found compared with the controls. In another study, muscle-derived stem cells were reported to possibly differentiate into bladder smooth muscle cells [8] and demonstrated contractile activity after seeding [20].

In our study, the induced obstructed bladder model showed a significant decrease in bladder wall thickness and maximum peak pressure but increased bladder weight with larger bladder capacities compared with normal bladder in control rats. In addition, structural changes of the detrusor muscle occurring during obstruction resulted in increased connective tissue-to-smooth muscle ratio and decreased SMA and smoothelin expression. Although BOO is a well-established procedure in rodents [21], the use of this model to induce hypocontractility remains challenging. In our hands, animals demonstrated a high variety in pathophysiological changes after 6 weeks of obstruction. This variability made it difficult to reach statistical significance, although all trends were in favour of cell treatment.

The functionality of regenerated smooth muscle bundles in treated and control animals was further investigated by cystometric analysis, which is the standard for functional evaluation of the lower urinary tract [22]. Our observation revealed significantly improved voiding pressures in the stem-cell-treated groups. Interestingly, the control rats after deligation, representing the transurethral resection of the prostate in patients, also demonstrated a level of natural healing capacities with partial recovery of contraction, which is not seen in patients with hypocontractile bladders in clinics.

We demonstrated that deligation and synchronous stem-cell treatment prevented further pathophysiological remodelling of the bladder wall, with histology showing the regrowth of smooth muscle bundles within the regenerated urinary bladder wall. Connective tissue-to-smooth muscle ratio was reduced in ADSCs and MPCs-treated groups compared with obstructed and deligated groups. When compared to the untreated group, the animals injected with either ADSCs or MPCs also showed a trend of lower collagen-to-smooth muscle ratio. The injected stem cells thus seem to have a supportive effect on improved remodelling with fast muscle regeneration.

In addition, we tracked the injected cells dispersed in dense muscle clusters. The recovered tissue architecture was confirmed by gene expression pattern and translation of important contractile proteins in cell-treated bladder tissue. Two major structural proteins in SMC, SMA and smoothelin were used to verify the regeneration of muscle tissue. SMA is an early and general marker of developing smooth muscle, but its expression is not



e

Maximum Peak Pressure

Samples	Mean ± SEM	P
Normal Bladder	45.92 ± 1.2	0.030
Obstruction	16.65 ± 5.8	-
ADSC	41.79 ± 8.4	0.039
MPC	42.51 ± 1.8	0.032
Deligation	29.2 ± 3	0.601

Average Slope

Samples	Mean ± SEM	P
Normal Bladder	1.5 ± 0.4	0.125
Obstruction	0.4 ± 0.3	-
ADSC	0.6 ± 0.2	0.954
MPC	1.7 ± 0.19	0.012
Deligation	1.0 ± 0.2	0.44

Fig. 4 Overview of the cystometric analysis with maximum peak pressure (**a**), threshold pressure (**b**), average slope (**c**) and voiding volume (**d**). **e** Summary tables of cystometric analysis with maximum peak pressure and average slope

restricted to smooth muscle. Smoothelin is a marker of the differentiated phenotype, and its expression is restricted to functional smooth muscle [23]. Deligated bladders treated with ADSCs and MPCs showed high levels of both SMA and smoothelin expression. This finding suggests that either paracrine factors from injected ADSCs and MPCs support smooth muscle regeneration or the injected cells formed new smooth muscle tissue [24]. As reported, the interaction of cell surface receptors, ligands and short-range-acting molecules between differentiated and undifferentiated cells significantly contribute to stem-cell differentiation [25].

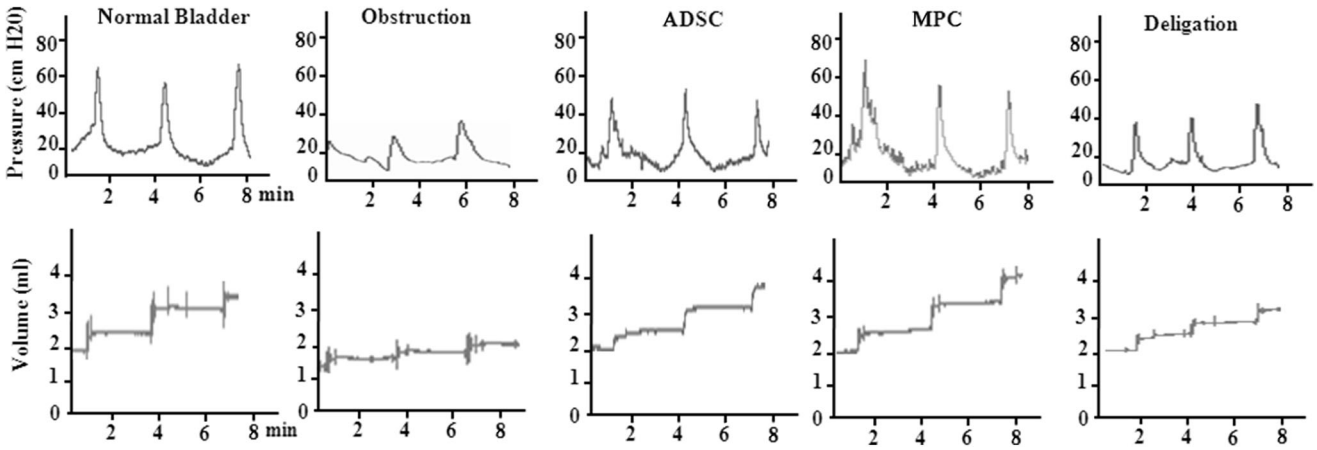


Fig. 5 Representative original cystometric traces with voiding volumes per group. Higher micturition pressures and improved voiding volumes are demonstrated after injection of ADSCs and MPCs compared with the obstructed and the deligated groups

Conclusion

We established a reliable small animal model for hypocontractile bladder and demonstrated that ADSCs and MPCs support the early restoration of BVD. Our results show improved voiding pressures and molecular expression of contractile proteins after cell therapy.

Acknowledgments We would like to thank Damina Balmer, M. Sc., for her assistance in the preparation of the manuscript. We are grateful to Irina Agarkova, PhD, for her support. This study was funded by Max und Hedwig Niedermaier-Stiftung and Fonds für Medizinische Forschung.

References

1. Roehrborn CG (2002) Etiology, pathophysiology, epidemiology, and natural history of benign prostatic hyperplasia. Campbell's Urol, Walsh PC, Retik AB, Vaughan EDJ, Wein AJ (eds), 8th ED, WB Saunders, Philadelphia:1297–1336
2. Fujita O, Asanuma M, Yokoyama T, Miyazaki I, Ogawa N, Kumon H (2006) Involvement of STAT3 in bladder smooth muscle hypertrophy following bladder outlet obstruction. Acta Med Okayama 60(6):299–309
3. Nitti VW, Kim Y, Combs AJ (1997) Voiding dysfunction following transurethral resection of the prostate: symptoms and urodynamic findings. J Urol 157(2):600–603
4. Lindroos B, Suuronen R, Miettinen S (2011) The potential of adipose stem cells in regenerative medicine. Stem Cell Rev 7(2):269–291. doi:10.1007/s12015-010-9193-7
5. Eberli D, Soker S, Atala A, Yoo JJ (2009) Optimization of human skeletal muscle precursor cell culture and myofiber formation in vitro. Methods 47(2):98–103
6. Partridge TA, Morgan JE, Coulton GR, Hoffman EP, Kunkel LM (1989) Conversion of mdx myofibres from dystrophin-negative to-positive by injection of normal myoblasts. Nature 337(6203):176–179. doi:10.1038/337176a0
7. Yiou R, Yoo JJ, Atala A (2003) Restoration of functional motor units in a rat model of sphincter injury by muscle precursor cell autografts. Transplantation 76(7):1053–1060. doi:10.1097/01.TP.0000090396.71097.C2
8. Yokoyama T, Huard J, Pruchnic R, Yoshimura N, Qu Z, Cao B, de Groat WC, Kumon H, Chancellor MB (2001) Muscle-derived cell transplantation and differentiation into lower urinary tract smooth muscle. Urology 57(4):826–831
9. Fullhase C, Soler R, Gratzke C, Brodsky M, Christ GJ, Andersson KE (2010) Spinal effects of the fesoterodine metabolite 5-hydroxymethyl tolterodine and/or doxazosin in rats with or without partial urethral obstruction. J Urol 184(2):783–789. doi:10.1016/j.juro.2010.03.104
10. Burmeister D, AbouShwareb T, D'Agostino R Jr, Andersson KE, Christ GJ (2012) Impact of partial urethral obstruction on bladder function: time-dependent changes and functional correlates of altered expression of Ca(2)(+) signaling regulators. Am J Physiol Renal Physiol 302(12):F1517–F1528. doi:10.1152/ajprenal.00016.2012
11. Hsiao ST, Asgari A, Lokmic Z, Sinclair R, Dusting GJ, Lim SY, Dilley RJ (2012) Comparative analysis of paracrine factor expression in human adult mesenchymal stem cells derived from bone marrow, adipose, and dermal tissue. Stem cells Dev 21(12):2189–2203. doi:10.1089/scd.2011.0674
12. Kolf CM, Cho E, Tuan RS (2007) Mesenchymal stromal cells. Biology of adult mesenchymal stem cells: regulation of niche, self-renewal and differentiation. Arthr Res Ther 9(1):204. doi:10.1186/ar2116
13. Albala DM, Lawson JH (2006) Recent clinical and investigational applications of fibrin sealant in selected surgical specialties. J Am Coll Surg 202(4):685–697
14. Yanagiuchi A, Miyake H, Nomi M, Takenaka A, Fujisawa M (2009) Modulation of the microenvironment by growth factors regulates the in vivo growth of skeletal myoblasts. BJU Int 103(11):1569–1573
15. Zhu WD, Xu YM, Feng C, Fu Q, Song LJ, Cui L (2010) Bladder reconstruction with adipose-derived stem cell-seeded bladder acellular matrix grafts improve morphology composition. World J Urol 28(4):493–498. doi:10.1007/s00345-010-0508-8
16. Huang YC, Shindel AW, Ning H, Lin G, Harraz AM, Wang G, Garcia M, Lue TF, Lin CS (2010) Adipose derived stem cells ameliorate hyperlipidemia associated detrusor overactivity in a rat model. J Urol 183(3):1232–1240. doi:10.1016/j.juro.2009.11.012
17. Zhao W, Zhang C, Jin C, Zhang Z, Kong D, Xu W, Xiu Y (2011) Periurethral injection of autologous adipose-derived stem cells with controlled-release nerve growth factor for the treatment of stress urinary incontinence in a rat model. Eur Urol 59(1):155–163. doi:10.1016/j.eururo.2010.10.038

18. Jankowski RJ, Deasy BM, Huard J (2002) Muscle-derived stem cells. Gene Ther 9(10):642–647. doi:10.1038/sj.gt.3301719
19. Nitta M, Tamaki T, Tono K, Okada Y, Masuda M, Akatsuka A, Hoshi A, Usui Y, Terachi T (2010) Reconstitution of experimental neurogenic bladder dysfunction using skeletal muscle-derived multipotent stem cells. Transplantation 89(9):1043–1049. doi:10.1097/TP.0b013e3181d45a7f
20. Lu SH, Cannon TW, Chermanski C, Pruchnic R, Somogyi G, Sacks M, de Groat WC, Huard J, Chancellor MB (2003) Muscle-derived stem cells seeded into acellular scaffolds develop calcium-dependent contractile activity that is modulated by nicotinic receptors. Urology 61(6):1285–1291
21. Melman A, Tar M, Boczko J, Christ G, Leung AC, Zhao W, Russell RG (2005) Evaluation of two techniques of partial urethral obstruction in the male rat model of bladder outlet obstruction. Urology 66(5):1127–1133. doi:10.1016/j.urology.2005.06.070
22. Alstrup K, Graugaard-Jensen C, Rittig S, Jorgensen KA (2010) Abnormal diurnal rhythm of urine output following renal transplantation: the impact of blood pressure and diuretics. Transplant Proc 42(9):3529–3536. doi:10.1016/j.transproceed.2010.08.028
23. Owens GK, Kumar MS, Wamhoff BR (2004) Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 84(3):767–801. doi:10.1152/physrev.00041.2003
24. Zhang R, Jack GS, Rao N, Zuk P, Ignarro LJ, Wu B, Rodriguez LV (2012) Nuclear fusion-independent smooth muscle differentiation of human adipose-derived stem cells induced by a smooth muscle environment. Stem Cells 30(3):481–490. doi:10.1002/stem.1023
25. Alexanian AR, Kurpad SN (2005) Quiescent neural cells regain multipotent stem cell characteristics influenced by adult neural stem cells in co-culture. Exp Neurol 191(1):193–197. doi:10.1016/j.expneurol.2004.10.006