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*Original Article*

# Seeding mesenchymal stem cells onto PLGA/collagen compound scaffold for bladder reconstruction in a rabbit model

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**Abstract:** Urinary bladder loss is one of the major problems due to congenital, trauma or malignancies. The traditional bladder reconstructive surgery with gastrointestinal segments has various complications. Therefore, we evaluated the alternative artificial bladder reconstructive surgery with seeding mesenchymal stem cells induced by Platelet-Derived growth factor-BB into PLGA/collagen compound scaffold in rabbit model. Total 21 male rabbits were randomly grouped: Group A (n=9), Group B (n=9), and Group C (n=3, control) with mesenchymal stem cells seeded into PLGA/collagen compound scaffold, small intestinal submucosa, and none, respectively. Bladder volume was tested preoperatively and at 4, 8, and 12 weeks postoperatively along with cystography at the same time. Additionally, H&E staining and immunohistochemistry with CD31 and smooth muscle-actin (α-SMA) monoclonal antibody were performed to observe regeneration of urothelium and smooth muscle cells. Our results exhibited a good biocompatibility with PLGA/collagen compound scaffold. The thickness of the grated segment were similar in Group A and B, close to native bladder tissue (Group C) at 12 weeks postoperatively. The bladder capacities in Group A were significantly better than that of the Group B (46.17±1.62 ml vs. 40.52±1.26 ml, $P<0.05$). Moreover, cystography revealed a better shape of reconstructed bladders in Group A. The histology and immunohistochemistry results of Group A demonstrated better well-regeneration of urothelium and smooth muscle cells, sustained positively for CD31 and α-SMA than that of Group B. In summary our study results suggested that seeding MSCs into PLGA/collagen compound scaffold promotes regeneration of the urothelium and smooth muscle cells in a rabbit model. Therefore, PLGA/collagen compound scaffold could be more suitable for bladder regeneration than SIS.

**Keywords:** Bladder reconstruction, tissue scaffold, mesenchymal stem cells, platelet-derived growth factor-BB, rabbit model

## Introduction

Trauma, tumors, congenital abnormalities and inflammation were the major reasons resulting in urinary bladder damage or loss. The traditional urinary bladder reconstruction methods of gastrointestinal segments pose a great challenge for urological surgeons with various complications, such as mucus production, leakage and ruptures, chronic bacteriuria, fibrosis, stone formation and so on [1]. Tissue-engineering techniques and autologous cell therapy involving implanting the biomaterial scaffolds seeded with applicable cells to repair or regenerate the damaged tissues have shown great potential in bladder repairing and reconstruction [2].

Theoretically, tissue engineering depended on autologous cells from the host organ. However, physiologically normal tissue might not always be accessible to elaborate a regenerative cystoplasty, leading to the use of stem cells in bladder reconstruction as an alternative. The aim of using stem cells is to promote the differentiation into urothelial cells and smooth muscle cells to maintain the normal histological bladder structure [3]. Mesenchymal stem cells (MSCs) derived from the bone marrow stroma have shown attractive potential for bladder reconstruction with their properties of easy isolation from a renewable source, self-renewal with a high proliferative capacity, low immunogenicity and differentiation into variety of tissue

types, such as urothelium, endothelial cells and smooth muscle cells [4].

Using biocompatible material has shown distinct benefits in the bladder reconstruction as cell delivery vehicles and for physically maintaining tissue replacement [4]. Till date, biomaterials are composed of 3 main categories and have been evaluated for bladder wall regeneration: 1) naturally derived materials such as collagen; 2) acellular tissue materials such as bladder submucosa; 3) synthetic materials such as poly lactic-co-glycolic acid (PLGA) [5]. However, there are no certain results clarifying the best scaffold for bladder wall regeneration of all the three layers [6]. The ideal scaffolds were considered to have both good compatibility and mechanical strength for easy handling and cell seeding. Thus, the idea of combining naturally derived polymers with synthetic polymers is promising in bladder regeneration.

Above all, in this study, we incorporated PDGF-BB with MSCs and then seeded into PLGA/collagen Compound scaffold to reconstruct the damaged bladder. Then, we evaluated the efficiency by functional and histological examination and discuss the potential mechanism.

## Materials and methods

### *MSCs isolation and culture*

MSCs were isolated and cultured from rabbits as previously described [7], approved by the Shanghai Fengxian Center Hospital Animal Care and Use Committee. In brief, bone marrow was aspirated from the sternum or the iliac crest of anaesthetized New Zealand White Rabbits, approximately 1.5~3.0 ml and diluted with 50 ml PBS containing preservative-free heparin (1000 U/mL). After centrifugation, 1500 r/min, 20 min at room temperature, the supernatant and adipose layer were discarded, and the cells were collected which was mixed with serum-free Dulbecco's modified Eagle's medium (DMEM). Then 20 ml bone marrow dilution was added to 20 ml Ficoll Lymphocyte separation medium to achieve a single nuclear cell. Then the single nuclear cells were diluted in DMEM with low glucose, L-glutamine, 110 mg/L Na-Pyruvate, pyridoxine HCl (GIBCO, Invitrogen, CA), fetal bovine serum (10% w/v; GIBCO) and incubated at 37°C with 5% humidified $CO_2$ for 24 hr. Then discarded the non-adherent cells and changed the medium every 2~3 days. When culture flasks became nearly confluent, the cells were detached and serially sub-cultured. Semiconfluent cells of fourth passage were co-cultured with 0.1 μg/L PDGE-BB for 7~14 days to be used for cell seeding experiments.

### *Preparing the PLGA/collagen compound scaffold*

The PLGA/collagen compound scaffold was prepared as previously described [8]. PLGA (Ethicon, Somerville, NJ) knitted mesh was immersed in a neutralized collagen solution and then incubated at 37°C for 2 hr. The gel formation was done during cell seeding.

### *Preparing the small intestinal submucosa (SIS)*

Sections of rabbit jejunum were harvested within 4 h of sacrifice. The fat was removed from rabbit's jejunum by washing carefully with water and cut into lengths of approximately 10 cm. Then, the tunica serosa and tunica muscularis were removed mechanically. The decellularized SIS was washed with hypertonic saline or detergent to remove the residual cells then immersed into 10% Neomycin sulfatesterilized solution for 20 min. The SIS was freeze-dried before use.

### *Seeding MSCs on scaffolds*

Fourth passage PDGE-BB induced MSCs were seeded into the outside of PLGA/collagen or SIS scaffolds by placing a cell suspension ($10^5$ cells/ml) into the matrix in a flask filled with DMED medium with low glucose, L-glutamine, 110 mg/L Na-Pyruvate, pyridoxine HCl and 10% FBS and incubated at 37°C with 5% humidified $CO_2$ for 7 days before implantation. The medium was changed daily.

### *Bladder reconstruction*

A total of 21 healthy New Zealand white male rabbits aged 3-5 months were selected and provided by Shanghai slack laboratory animal co., LTD. The selected rabbits were randomly divided into three groups: Group A (n=9), Group B (n=9), and Group C (n=3, control). Under anesthesia, a defect was created in the dome of the bladder wall by surgical incision in 18 rabbits (A and B), representing approximately 1×1 $cm^2$. The seeded PLGA/collagen compound Nanofiber scaffold, and SIS scaffold were sutured as patch into the dome of bladder with

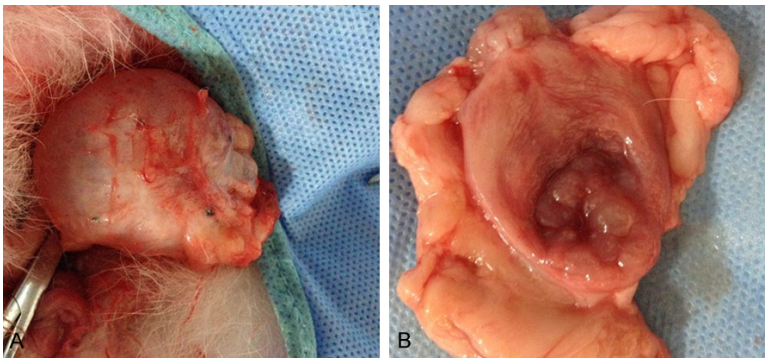


**Figure 1.** A. No diverticulum on the implanted portions covered by soft, vascularized connective tissue was shown in the Group A and B. B. None stone formation was formed in Group A and B.

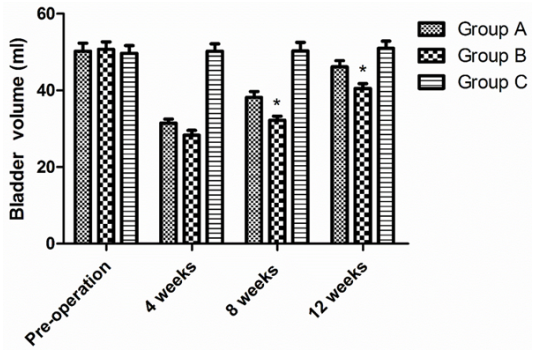


**Figure 2.** Bladder volume was evaluated at pre-operation time and at 4, 8, 12 weeks after surgery in the three groups. Group A: PLGA/collagen compound scaffold, Group B: SIS, Group C: sham operation control. *$P<0.05$, Group A *vs.* Group B.

continuous 5-0 Vicryl suture in Group A and Group B, respectively. Nothing was done in Group C, Sham operation. Then all the rabbits in Group A and B were placed with Prolene marking sutures to rule out variation in the tissue harvest. Finally, saline solution was instilled into the bladder to test for any leakage.

*Measurement of bladder volume*

Bladder volume was tested by a 7 Fr double-lumen transurethral catheter. The bladder was emptied and then was filled with pre-warmed saline solution at a constant rate. Maximal capacity of the bladder was defined as the volume of infusion that triggered the first leakage of urine [9].

*Histology and immunohistochemistry*

Three rabbits in each group were killed separately at 4, 8, and 12 weeks after implantation. Before being euthanized, cystography was performed and the capacity of the bladder was measured. Then bladder tissues were isolated immediately after euthanization and fixed in 10% neutral buffered formalin. 5 um sections of paraffin wax-embedded tissue was sustained with H&E and immunohistochemistry staining. The urothelial cell layers were identified using the CD31 monoclonal antibody (Sigma, St. Louis, MO, USA) and smooth muscle cells at the repaired site were identified by α-smooth muscle actin (α-SMA) monoclonal antibody (Sigma, St. Louis, MO, USA).

*Statistical analysis*

The data of each group was expressed with Mean ± SD. The difference of each group regarding the urodynamic studies was carried out by one-way ANOVA, followed by the LSD post-hoc test within two groups' comparison using Prism 3.0 software (GraphPad Software Inc. USA). $P< 0.05$ was defined as a statistical significance.

## Results

*Macroscopic features*

The total of 21 rabbits survived till being killed. Then the rabbits were anesthetized and killed/terminated as previously described [9]. The bladder was exposed through a midline incision for macroscopic inspection. It is easy to identify and distinguish the irresolvable reference sutures at every time point. At 4 weeks postoperatively, there was no diverticulum on the transplanted portions and the outer surface of the reconstructed bladder was covered by/with soft, vascularized connective tissue. At 12 weeks postoperatively, the thickness of the grated/grafted segments were similar in Group A and B, and close to native bladder tissue (Group C). None stone formation was shown in all the rabbits in Group A and B (**Figure 1**).

*Urodynamic studies*

As shown in **Figure 2**, preoperatively, the bladder capacities showed no significant difference among the three groups (Group A:

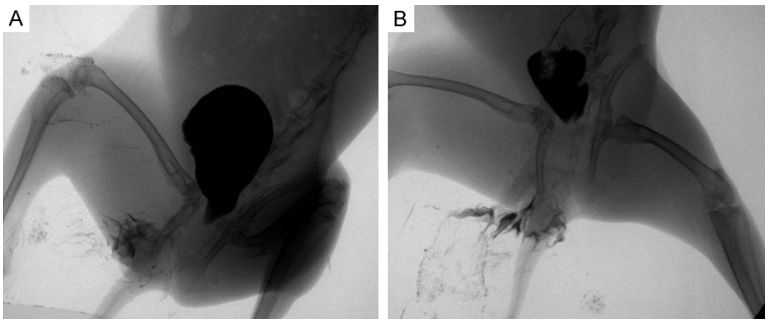


**Figure 3.** Bladder Cystography performed at 12 weeks postoperatively. A. PLGA-collagen hybrid scaffold. B. SIS.

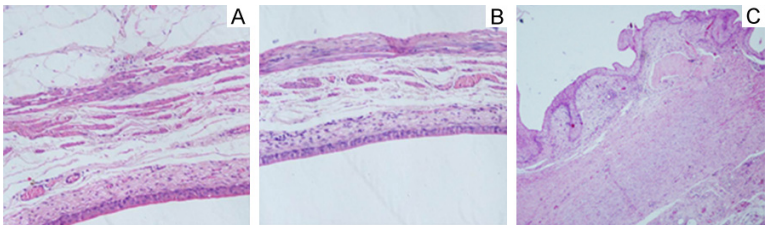


**Figure 4.** Histological features of the transplanted grafts at 12 weeks postoperatively. A. PLGA-collagen hybrid scaffold. B. SIS. C. Sham operation control.

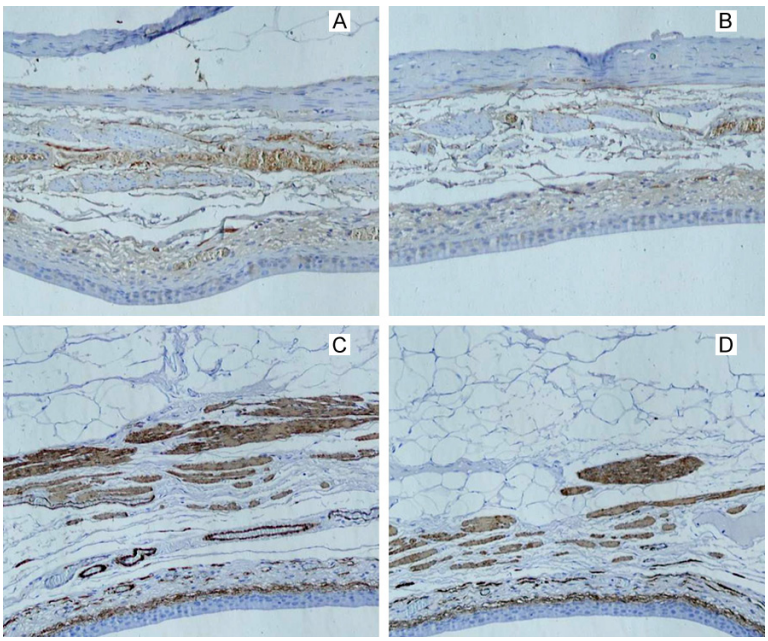


**Figure 5.** Immunohistochemistry of the transplanted grafts. A. Staining with CD31 (Group A), B. Staining with CD31 (Group B), C. Staining with α-SMA (Group A), D. Staining with α-SMA (Group B).

50.23±2.12 ml, Group B: 50.73±1.93 ml, and Group C: 49.68±2.01 ml). At 4 weeks after surgery, the bladder capacities were decreased to 31.48±1.03 ml in Group A and 28.32±1.27 ml in Group B, showing no statistical significance ($P > 0.05$), and Group C was 50.21±1.98 ml. However, at 8 and 12 weeks after surgery, bladder capacities in Group A were significantly better than that of the Group B (38.16±1.54 ml and 46.17±1.62 ml vs. 32.21±1.08 ml and 40.52±1.26 ml respectively, $P<0.05$). Meanwhile, at the same two time points, the bladder capacity of Group C showed no significant difference (50.31±2.21 ml and 50.98±1.89 ml, $P > 0.05$). Furthermore, cystography revealed a better shape of reconstructed bladders in Group A (**Figure 3**).

*Histology and immunohistochemistry*

The bladder tissue was studied by histology and immunohistochemistry. At 4 weeks postoperatively, the H&E results showed multilayered urothelium at the implanted sites and smooth muscle cells around the graft site, both in Group A and B. At 12 weeks, in Group A, the reconstructed bladder demonstrated a similar appearance to the native bladder tissue histologically in Group C. However, in Group B, the smooth muscle cells at the graft site were organized, but still with significant difference from the native bladder tissue in Group C (**Figure 4**). Additionally, immunohistochemical staining was positive for α-smooth muscle actin (α-SMA) in Group A and B, meaning that smooth muscle cells were well differentiated. The entire urothelial cell layers stained positively with anti-CD31 in Group A and B (**Figure 5**).

## Discussion

Bladder augmentation or partial substitution is often needed in bladder damage, like neurogenic bladder, bladder exstrophy, oncology, and

so on. The essential task is to re-construct the function of the bladder. The traditional bladder reconstruction using the gastrointestinal tract leads to several complications including metabolic, infection, perforation, urolithiasis, disturbances and malignant transformation [2, 6]. However, the ideal surgical approach is considered when there is low complication rate and good functional result maintaining bladder capacity and compliance. Recently, tissue engineering has become a new hope for bladder reconstruction as a viable alternative to avoid many of these complications.

Over the decades, a number of investigators have researched the functional regeneration of bladder and urethra defects by seeding cells into biodegradable materials and the results showed that a cell-seeded scaffold is an alternative way to successful repair of bladder and urethral defects. Sharma AK et al. [10] evaluated the efficiency of seeding the bone marrow derived mesenchymal stem cells (BMSCs) on the small intestinal submucosa (SIS) to reconstruct the bladder in a baboon model. The results confirmed the BMSCs-seeded SIS on bladder reconstruction with typical bladder architecture, muscle-to collagen ratios of 32% vs. 52% in unseeded vs. seeded group, respectively, and a greater bladder capacity.

As seeding cells and scaffolds were the most important two elements in tissue engineering. A lot of researches were conducted on exploring the proper seeding cells or scaffolds. Traditionally, autologous bladder smooth muscle and urothelial cells are seeded into acellular matrices or synthetic scaffolds [11, 12]. However, these cells were difficult to harvest and culture in vitro. Then investigators are focusing on the other alternative cell sources, such as embryonic stem cells, muscle-derived cells, adult stem cells and bone marrow stem cells (BMSCs) [13]. Mesenchymal stem cells (MSCs) derived from the bone marrow stroma have been confirmed to differentiate into various tissue types in vitro, such as smooth muscle urothelium and endothelial cells with the advantage of easy to achieve, adequate resources, less traumatic, no rejection response and strong reproductive activity [14], and considered to be an attractive candidate for bladder reconstruction [15].

The standard augmentation matrix or scaffold that can afford almost constant pressure values while suiting large changes in intravesical volume is still controversial. Small intestinal submucosa (SIS) and bladder acellular matrix grafts (BAMGs) were most often investigated in animal models and achieved satisfactory results, especially SIS, which could support the growth of urothelial cells and the regeneration of urethra without immune response. However, there still exist lacks of enough growth of urothelia and smooth muscle cells, leading to poor contractile function [16]. Thus, the study of bladder alternative material seems important for bladder augmentation or substitution. Recently, the most commonly used materials in bladder regeneration research were Teflon, collagen matrices and silicon [17]. However, the major restriction of them is the incompatibilities between cell and tissue, thus/hence leading to the development of other synthetic polymers, like Poly (lactic-co-glycolic acid) (PLGA), one of the most successfully developed biodegradable synthetic polymers [4]. PLGA has shown its great potential in bladder regeneration with several advantages, such as biocompatibility and biodegradability [18]. Additionally, collagen has been often used in tissue engineering because of its ability to be easily manipulated, none immune response and biocompatibility and has been considered to be the ideal material for nanofiber scaffold [19]. Thus, we assume that the combination of PLGA and collagen will make the scaffold to have the character of both mechanical property and biocompatibility and might be an ideal alternative material to bladder functional reconstruction.

In the present study, we successfully isolated MSCs from rabbits and prepared the PLGA/collagen compound nanofiber scaffold. As is well known, bioactive factors also play an important role in bladder reconstruction with the essential signal and regulatory functions in the regeneration and development, maintenance of bladder tissues. Adding the exogenous delivery of bioactive factors to make up the deficiency of endogenous bioactive factors was important for tissue regeneration and was considered as having critical role in bladder regeneration [20]. Platelet-derived growth factor-BB (PDGF-BB) was reported as a mitogenic signaling stimulator in urinary tract smooth muscle cell. It has been reported that incorporating PDGF-BB into BAM can enhance bladder reconstruction by functional and histological examination [1]. In our study, we co-administrated Platelet-Derived

Growth Factor-BB (PDGF-BB) with MSCs and investigated its role in bladder smooth muscle regeneration and vascularization. Then we seeded the induced MSCs into the PLGA/collagen compound scaffold to reconstruct bladder in rabbit model (Group A). We also performed SIS as positive control (Group B) and sham group as blank control (Group C). The results, as described above showed that MSCs demonstrate good biocompatibility with PLGA/collagen compound scaffold. Seeding the PDGF-BB induced MSCs into the PLGA/collagen compound scaffold can promote the regeneration of bladder smooth muscle and urothelial cells which is apparently superior to SIS scaffold. These results indicated that PLGA/collagen compound scaffold might be an excellent scaffold material for bladder augmentation or partial substitution.

## Conclusions

Based on our study results, seeding MSCs co-administered with PDGF-BB into PLGA/collagen compound scaffold can promote better regeneration of urothelium and smooth muscle cells than seeding into SIS in a rabbit model. Therefore, we can conclude that PLGA/collagen compound scaffold might be more suitable for bladder regeneration than SIS.

## Disclosure of conflict of interest

None.

## Abbreviations

MSCs, Mesenchymal stem cells; PDGF-BB, Platelet-Derived Growth Factor-BB; PLGA, poly lactic-co-glycolic acid; α-SMA, α smooth muscle-actin; CD31, Platelet endothelial cell adhesion molecule-1; DMEM, Dulbecco's modified Eagle's medium; SIS, small intestinal submucosa; BMSCs, bone marrow derived mesenchymal stem cells; BAMGs, bladder acellular matrix grafts; hr, hour.

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## References

[1] Zhou L, Yang B, Sun C, Qiu X, Sun Z, Chen Y, Zhang Y and Dai Y. Coadministration of platelet-derived growth factor-BB and vascular endothelial growth factor with bladder acellular matrix enhances smooth muscle regeneration and vascularization for bladder augmentation in a rabbit model. Tissue Eng Part A 2013; 19: 264-276.

[2] Yuan H, Zhuang Y, Xiong J, Zhi W, Liu L, Wei Q and Han P. Human umbilical mesenchymal stem cells-seeded bladder acellular matrix grafts for reconstruction of bladder defects in a canine model. PLoS One 2013; 8: e80959.

[3] Panda A. Tissue engineering and stem cell research in urology: is the moment yet to come? Indian J Urol 2015; 31: 87-88.

[4] El-Taji OM, Khattak AQ and Hussain SA. Bladder reconstruction: the past, present and future. Oncol Lett 2015; 10: 3-10.

[5] Atala A. Tissue engineering of human bladder. Br Med Bull 2011; 97: 81-104.

[6] Kajbafzadeh AM, Tourchi A, Mousavian AA, Rouhi L, Tavangar SM and Sabetkish N. Bladder muscular wall regeneration with autologous adipose mesenchymal stem cells on three-dimensional collagen-based tissue-engineered prepuce and biocompatible nanofibrillar scaffold. J Pediatr Urol 2014; 10: 1051-1058.

[7] Sahoo S, Toh SL and Goh JC. PLGA nanofiber-coated silk microfibrous scaffold for connective tissue engineering. J Biomed Mater Res B Appl Biomater 2010; 95: 19-28.

[8] Ajalloueian F, Zeiai S, Fossum M and Hilborn JG. Constructs of electrospun PLGA, compressed collagen and minced urothelium for minimally manipulated autologous bladder tissue expansion. Biomaterials 2014; 35: 5741-5748.

[9] Zhu WD, Xu YM, Feng C, Fu Q and Song LJ. Different bladder defects reconstructed with bladder acellular matrix grafts in a rabbit model. Urologe A 2011; 50: 1420-1425.

[10] Sharma AK, Bury MI, Marks AJ, Fuller NJ, Meisner JW, Tapaskar N, Halliday LC, Matoka DJ and Cheng EY. A nonhuman primate model for urinary bladder regeneration using autologous sources of bone marrow-derived mesenchymal stem cells. Stem Cells 2011; 29: 241-250.

[11] Yoo JJ, Meng J, Oberpenning F and Atala A. Bladder augmentation using allogenic bladder submucosa seeded with cells. Urology 1998; 51: 221-225.

[12] Becker C and Jakse G. Stem cells for regeneration of urological structures. Eur Urol 2007; 51: 1217-1228.
[13] Zhu WD, Xu YM, Feng C, Fu Q, Song LJ and Cui L. Bladder reconstruction with adipose-derived stem cell-seeded bladder acellular matrix grafts improve morphology composition. World J Urol 2010; 28: 493-498.
[14] Chen S, Zhang HY, Zhang N, Li WH, Shan H, Liu K and Yang Y. Treatment for chronic ischaemia-induced bladder detrusor dysfunction using bone marrow mesenchymal stem cells: an experimental study. Int J Mol Med 2012; 29: 416-422.
[15] da Silva Meirelles L, Chagastelles PC and Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 2006; 119: 2204-2213.
[16] Wu S, Liu Y, Bharadwaj S, Atala A and Zhang Y. Human urine-derived stem cells seeded in a modified 3D porous small intestinal submucosa scaffold for urethral tissue engineering. Biomaterials 2011; 32: 1317-1326.
[17] Matoka DJ and Cheng EY. Tissue engineering in urology. Can Urol Assoc J 2009; 3: 403-408.
[18] Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A and Preat V. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release 2012; 161: 505-522.
[19] Muthusubramaniam L, Peng L, Zaitseva T, Paukshto M, Martin GR and Desai TA. Collagen fibril diameter and alignment promote the quiescent keratocyte phenotype. J Biomed Mater Res A 2012; 100: 613-621.
[20] Tayalia P and Mooney DJ. Controlled growth factor delivery for tissue engineering. Adv Mater 2009; 21: 3269-3285.