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Continued sustained release of VEGF by PLGA nanospheres modified BAMG stent for the anterior urethral reconstruction of rabbit

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ABSTRACT

Objective: To study the biocompatibility and neovascularization of the PLGA nanospheres wrapped with vascular endothelial growth factor (VEGF), which can improve bladder acellular matrix graft (BAMG) with local continuous release of VEGF. **Methods:** A total of 18 rabbit model (length of stenosis: 3cm) with anterior urethral stricture were used as experimental animals and divided into three groups. Group A as the control group: Simple BAMG scaffold materials for urethral reconstruction. Group B as the blank group: PLGA microspheres modified BAMG for urethral reconstruction. Group C: PLGA conjugated with VEGF and modified BAMG for the urethral reconstruction. All rabbits underwent urethral angiography after 7 days, 15 days, 1 month and 3 months after the operation, and one rabbit in each group was sacrificed to be prepared for the organization histologic examination, HE staining, masson staining, CD31, 34 and a-SAM immunohistochemical detection in the repaired sites. **Results:** In group A, significant urethral restenosis occurred in two rabbits after 15 days of the operation, HE and masson staining showed a lot of collagen arranged in the repaired sites, and there were a large number of inflammatory cell infiltration, and there were also CD31, 34 in the repaired sites. a-SAM microvascular tag count showed a small amount of microvascular; Group B showed anastomotic restenosis, HE and masoon staining showed inflammatory cell infiltration and collagen deposition; Group C: urethrography showed lumen patency. There were a small amount of inflammatory cell infiltration after 7 and 15 days after the operation, and there were also CD31, 34 in the repaired sites. The a-SAM microvascular tag count showed many microvascular. And the difference was significant. **Conclusions:** Anterior urethral reconstruction with sustained-release of VEGF by PLGA nanospheres modified BAMG stents can reduce postoperative restenosis. It can also reduce collagen deposition and scar formation, promote angiogenesis of the repair tissue; therefore it in valuable in the tissue-engineered urethral reconstruction.

1. Introduction

Reconstruction of the urinary tissue defects has always been the challenge and some researches focus on the urological surgeon. The traditional autologous extraction method of non-urinary system tissues always brings new trauma to the body, the therapeutical effect is not ideal, and usually accompanied by serious complications[1]. The development of tissue engineering technology provides a new therapeutic approach to the reconstruction for the urinary tract tissue defects. The urethral alternative restorative materials become possible, and the key to a successful treatment focuses on the blood supply in the repaired sites[2]. In this study, the bladder acellular matrix graft (BAMG) was used as a stent for the reconstruction of the urinary in order to change the anterior urethral of the

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rabbit urethral defect model.

2. Materials and methods

2.1. Materials and main reagents

A total of 18 healthy New Zealand white male rabbits aged 3-5 months were selected, and the animals were provided by the Experimental Animal Center of our hospital. These animals were then randomly divided into three groups, with six white rabbits in each group. Group A, the control group had simple BAMG scaffold materials to repair the urethra; Group B: Blank PLGA microspheres modified BAMG for urethral reconstruction; Group C: PLGA conjugated with VEGF and modified BAMG for the urethral reconstruction. The vascular endothelial growth factor (VEGF) reagents, CD31, 34 and a-SAM immunohistochemistry kit were purchased from Wuhan Boster Biological Engineering Co., Ltd.; The Masson trichrome kits were purchased from Shanghai Rong Bo Biotechnology Co., Ltd.; HE staining kit was provided by our laboratory. Polylactic acid-glycolic acid (PLGA) were purchased from Jinan DaiGang Biotechnology Co., Ltd. ; Dimethyl sulfoxide, polyvinyl alcohol: Sigma Company; Methylene chloride, sodium chloride, analytical grade: Zhengzhou Honglida Chemical Co., Ltd.

2.2. Methods

Preoperative preparation and anesthesia: After fasting for 12 h and water deprivation for 4 h before the operation, 100 000 U penicillin were given to the white rabbit 30 min preoperative by intramuscular injection (2-3 times a day). The pentobarbital sodium was formulated as 3% saline solution, 2 mL sodium pentobarbital solution were injected slowly through ear vein for anesthesia. After effective anesthesia, animals were fixed to the operating table at prone position, and animals' heads were fixed with the head frame. The damaged urethra was dissected and fully exposed. BAMG scaffold material which prepared by different methods of each group were placed in the repaired sites. Its ends were continuously sutured to the urethral stump with the 0/6 DG thread and then formed the urethra. The muscle, deep fascia and skin were closed layer by layer after hemostasis. All rabbits underwent urethral angiography after 7 d, 15 d, 1 month and 3 months after the operation, and one rabbit in each group was sacrificed to be prepared for the histological examination, HE staining, masson staining, CD31, 34 and a-SAM immunohistochemical detection in the repaired sites. It was analyzed by (Image Pro plus 4.02) multimedia color pathological image software. Positive staining and the background were automatic measured by gray level transformation respectively. For different regions, five non-overlapping high-power fields in this experiment was selected to analyze the positively stained area and the vision of the total area by percentage and an average was applied.

2.3. Effect evaluation

If diameter of the repaired urethral was $\leqslant$80% diameter of the original urethral, it was regarded as urethral stricture. The immunohistochemistry artificial count analysis of pathological diagnosis was performed. The CD31, CD34 and a-SAM-positive reactants revealed from light brown to dark brown particles under the light microscope.

2.4. Statistical methods

Data in every group were expressed with Mean$\pm$SD. The differences between the groups were analyzed by one-way ANOVA.SNK test was used to compare among groups. $P<0.05$ was regarded as statistical significance.

3. Results

3.1. Pathological morphology observation

Group A: No obvious urethral restenosis was observed in the animals 7 d after the operation, HE and masson stain showed slight fibrosis in the repaired sites, and there was a large number of inflammatory cell infiltration. Reexamination after 15 after of the operation showed there was significant urethral restenosis in two rabbits, a lot of arranged collagen and there were obvious infiltration in inflammatory cells. Reexamination one month after operation showed the degree of fibrosis had increased and the inflammatory cell infiltration was significantly reduced. Reexamination three months after operation showed obvious fibrosis and a small amount of inflammatory cell infiltration. The pathological changes of group B were similar to group A. The HE and masson stain showed the infiltration of inflammatory cells and the collagen deposition, reexamination 15 d after the operation showed the anastomotic restenosis in two rabbits. In group C, postoperative examination after 7, 15 days showed no obvious urethral restenosis among the animals. The degree of stenosis was significantly higher than group A and group B (Table 1), a few of infiltration of inflammatory cells were observed. Reexamination one month after operation showed slight fibrosis and a small amount of inflammatory cell infiltration. Reexamination after three months showed no obvious urethral restenosis and a few of

infiltration of the inflammatory cells.

Table 1

Degree of restenosis for the urethra of the white rabbit (%).

Groups	7 d after operation (n=6)	15 d after operation (n=5)	1 month after operation (n=4)	3 months after operation (n=3)
Group A	96.5±2.4	83.2±5.3	81.6±4.6	78.6±3.6
Group B	96.8±2.2	81.5±4.2	80.1±4.0	79.3±3.8
Group C	99.3±0.5	99.0±0.4*	98.6±0.6*	98.6±0.6*

*Compared with group A, P<0.05.

3.2. CD31, 34, a-SAM expression in urethral tissue

Positive staining of CD31 protein were expressed in all microvascular endothelial cells of the granulation tissue, which was located in the cytoplasm. The expression of it in group C was significantly increased compared with group A and group B (P<0.05). Positive staining of CD34 protein were expressed in vascular endothelial cells and located in the cytoplasm. The expression in group C was significantly increased compared with group A and group B (P<0.05). Positive staining of a-SAM protein were expressed in vascular smooth muscle cells and located in the cytoplasm. The expression of it in group C was significantly increased compared with group A and group B (P<0.05) (Table 2).

Table 2

Immunohistochemical expression of CD31, 34 a-SAM of white rabbits (mean±sd) (%).

Groups	CD31	CD34	a-SAM
Group A	3.1±0.7	0.4±0.1	6.5±0.5
Group B	3.0±0.7	0.5±0.1	6.1±0.6
Group C	11.5±2.2*	5.5±0.9*	0.4±0.1*

*Compared with group A, P<0.05.

4. Discussion

The most important factors of tissue engineering includ seed cells and scaffold. Currently the urethral acellular matrix, BAMG and small intestinal submucosa are the main sources of material for urinary reconstruction. Because BAMG comes from the urinary system, it has good histocompatibility, and as the membrane materials, BAMG can also repair defects according to the shape of the urethra. Therefore, the study on BAMG stent receives much concern. However, urethral stricture is the main factor for the poor treatment. The systemic therapy fails to achieve a satisfactory effect for the urethral stricture, and is also with systemic complications. A large number of studies suggest that reducing the local inflammatory response and increasing the local blood supply are the main direction to solve this problem, therefore the nanocarrier material PLGA which has a good bio-security has gained much attenlion[3]. This material is a synthetic biodegradable polymer, which causes no side effect in humans. Drugs can be released more slowly by it, which is more conducive to maintain drug concentration and guarantee the treatment effect.

In the study of angiogenesis, the commonly used endothelial cell markers are CD31 and CD34. Previous studies have found that CD31 can be expressed on vascular endothelial cells, platelets, macrophages and neutrophils, and plays an important role in inflammatory angiogenesis[4]. CD34 is often expressed in hematopoietic stem cells, endothelial cells, and embryonic mesenchymal cells, which can accelerate the vascular buds endothelial cell migration in the process of capillary formation[5]. In this study, the expression of CD31 and CD34 were significantly increased in white rabbit which underwent persistent release VEGF treatment. There were apparent edema and a large number of capillary proliferations at the two junctions in the urethral repaired sites. Considering the vascular endothelial growth factor belong to the family of platelet-derived growth factor, and as a specific mitosis of the arteriovenous and lymphatic endothelial cells, it can not only promote the 3D angiogenesis in vitro, but also promote the division of capillary endothelial cell and plasminogen glial enzymes and vasoactive substances[4].

Although the mechanism of VEGF and CD31, CD34 is still not clear, we believe that the VEGF still has no species specificity. It can stimulate the release of vasoactive substances of vascular endothelial cell, thus can facilitate the growth and repair of tissue. That is consistent with the related literatures[6,7]. a-SAM is the most important actin microfilaments in the smooth muscle cell, which is mainly related to the degree of tissue damage and played an important role in the mechanism of tissue fibrosis[8,9]. This study showed that after VEGF treatment, a-SAM protein expression of the repair urethral tissue was significantly reduced, thus can effectively avoid the urethral stricture which is caused by fibrosis in the repaired sites. Studies have shown that a-SAM can be used as early predictors of the chronic fibrosis[10]. We think that the mechanism of therapeutic effect of VEGF may be that it effectively inhibit fibrosis by improving tissue ischemia and hypoxia, thereby preventing the stricture in the repaired sites of the urethra.

In summary, we use BAMG as the scaffold material in this study, and performed the anterior urethral reconstruction by increasing the PLGA nanospheres which can sustained-release VEGF, thus can also reduce restenosis after the operation. We initially established a construction method of a nice tissue-engineered urinary tract epithelial tissue in

vivo. Because this method has the multiple effects including promote angiogenesis and reduce scarring , we think there will be broad prospects for the applications of tissue-engineered urethra reconstruction.

Conflict of interest statement

We declare that we have no conflict of interest.

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