A Nanomedicine Approach to Effectively Inhibit Contracture During Bladder Acellular Matrix Allograft-Induced Bladder Regeneration by Sustained Delivery of Vascular Endothelial Growth Factor

Qianwei Xiong, MD,^{1,*} Houwei Lin, MD,^{1,*} Xiaolin Hua, MD,¹ Li Liu, PhD,² Ping Sun, MD,¹ Zhen Zhao, PhD,² Xiaowei Shen, MD,¹ Daxiang Cui, PhD,³ Maosheng Xu, MD,¹ Fang Chen, MD, PhD,⁴ and Hongquan Geng, MD, PhD¹

Macroscopic evidence of contracture has been identified as a major issue during the regeneration process. We hypothesize that lack of angiogenesis is the primary cause of contracture and explore a nanomedicine approach to achieve sustained release of vascular endothelial growth factor (VEGF) to stimulate angiogenesis. We evaluate the efficacy of poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) for long-term (3 months) sustained release of VEGF in bladder acellular matrix allografts (BAMA) in a swine model. We anticipate that the sustained release of VEGF could stimulate angiogenesis along the regeneration process and thereby inhibit contracture. Bladder was replaced with BAMA (5 × 5 cm), modified with PLGA NPs encapsulated with VEGF in a pig model. The time points chosen for sampling were 1, 2, 4, and 12 weeks. The regenerated areas were then measured to obtain the contracture rate, and the extent of revascularization was calculated using histological and morphological features. In the control group of animals, the bladder was replaced with only BAMA. The *in vivo* release of VEGF was evident for ~3 months, achieving the goal of long-acting sustained release, and successfully promoted the regeneration of blood vessels and smooth muscle fibers. In addition, less collagen deposition was observed in the experimental group compared with control. Most importantly, the inhibition of contracture was highly significant, and the ultimate contracture rate decreased by ~57% in the experimental group compared with control. In isolated strips analysis, there were no significant differences between BAMA-regenerated (either VEGF added or not) and autogenous bladder. BAMA modified with VEGF-loaded PLGA-NPs can sustainably release VEGF *in vivo* (>3 months) to stimulate angiogenesis leading to the inhibition of contracture. This is the first study to report a viable nanomedicine-based strategy to overcome contracture during bladder regeneration induced by BAMA. Furthermore, this study also confirms that insufficient angiogenesis plays a crucial role in the onset of contracture.

Introduction

TISSUE ENGINEERING has seen incredible growth in the last decade and now finds a broad range of clinical applications especially in stem cell-based regenerative medicine and in reconstruction of tissues such as skin, breast, and cartilage.^{1,2} Contracture is a common side effect of the regeneration process. The successful use of a large segment (>24 cm²) of porcine bladder acellular matrix allograft (BAMA) for bladder regeneration has been documented in animal models in

the past.³ This seminal work showed the technical feasibility of BAMA implantation, which may provide a viable surgical option to bladder augmentation. However, the technique can be particularly serious when replacing the bladder with BAMA can severely reduce the curative effect of alternation. Some studies reported that the average contracture rate is ~48%,³ and so, contracture is a basic problem that has restricted the viable clinical applications of this technique. Insufficient angiogenesis is considered to be an important cause of contracture.⁴ Previous studies have replenished the growth factors in a

¹Department of Pediatric Urology, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China.

²Shanghai Institute of Pharmaceutical Industry, Shanghai, People's Republic of China.

³Key Laboratory for Thin Film and Microfabrication of Ministry of Education, Department of Bio-Nano Science and Engineering, Institute of Mic-Nano Science and Technology, Shanghai Jiao Tong University, Shanghai, People's Republic of China.

⁴Department of Urology, Children's Hospital affiliated to Shanghai Jiao Tong University, Shanghai, People's Republic of China.

*These authors contributed equally to this work and should be regarded as first joint authors.

porous collagen scaffold, using several methods, including rehydration, hyaluronic acid, to improve revascularization.^{5–9} Kanematsu *et al.* pioneered in this approach where he first rehydrated the basic fibroblast growth factor (bFGF) and then incorporated it into the BAMA. In his method, because the bFGF just released for about 3 weeks and stopped, the regeneration was improved only for a short period of time when the bFGF was present. Youssif's group observed the same even with a different incorporation approach, simply because the period of growth factor release was still short (2–3 weeks). Although Loai *et al.* attempted to tackle the problem by first dissolving the vascular endothelial growth factor (VEGF) into hyaluronic acid, they also failed, because the hyaluronic acid filled the holes of the scaffold, a texture essential for bladder regeneration.⁸ Since the release cycle of growth factors *in vivo* is only 2–3 weeks, none of these methods achieved a long-term sustained release. In addition, results showed that, while contracture was inhibited for a short span (2–3 weeks) after implantation, a significant long-term improvement was not seen. We hypothesize that angiogenesis must be enhanced during the entire process of tissue regeneration to be able to suppress contracture effectively. A long-term sustained release system of VEGF-encapsulated poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) was previously reported from our laboratories. The thermoresponsive Pluronic F127 system was used for the incorporation of NPs into BAMA. *In vitro* studies confirmed that sustained release of VEGF could be achieved in BAMA for 2–3 months.¹⁰ In this follow-up study, we used VEGF NPs in a modified BAMA alternative bladder and determined whether the sustained release of VEGF could effectively restrain contracture.

Our approach aimed to stimulate angiogenesis using long-term sustained release of VEGF and thus inhibit contracture. We believe that this will be a novel and effective method to resolve the problem of contracture during tissue engineering.

Materials and Methods

The preparation of PLGA-NPs and their incorporation into BAMA

The synthesis of VEGF-encapsulated PLGA NPs followed a previously reported procedure from our laboratories.¹⁰

Briefly, PLGA NPs containing VEGF (0.1 µg per mg of NPs) were prepared using the double emulsion solvent evaporation technique. As-synthesized NPs were recovered in a lyophilized form and then resuspended in distilled water. The suspension was added to the F127 solution to reach a final F127 concentration of 25% w/v. Finally, the VEGF-PLGA-NPs were sequentially injected into the BAMA to form a homogeneous mixture and maintained at 37°C.¹⁰

The surgical process

Twenty-four female pigs were randomly divided into experimental and control groups. In the experimental group, a 2 µg VEGF-PLGA-NP was incorporated into BAMA (5 × 5 cm) using Pluronic-F127, a temperature-sensitive gel system to replace the bladder. In the control group, BAMA alone was used. Animals were not allowed food or water for 24 h before the surgery. Pigs were anesthetized using ketamine and pentobarbital, and a midline lower abdominal incision followed by partial cystectomy (35–50% of the

bladder) was performed. Continuous 6/0 absorbable polydioxanone sulfate sutures were used. The margins of BAMG were marked using unabsorbable silk sutures at each quadrant to identify the future graft site. The integrity of the sutures was tested by sodium chloride instillation, and the abdominal wall and skin were closed and monitored. All animals received intramuscular penicillin for 5 days after surgery. Three pigs in each group were sacrificed at each time point of week 1, 2, 4, and 12 postimplantation (Fig. 1).

Determination of the contracture rate of BAMA

Piglets were sacrificed at 4 and 12 weeks and their entire bladders were removed and maintained under flaccid conditions. AUTOCAD software was then used to measure the bladder regeneration acreage, which was identified with nonabsorbed sutures. The contracture rate was calculated based on the equation: (the area of original BAMA – the area of regenerated bladder)/the area of original BAMA.

Histological structure after staining

At weeks 1, 2, 4, and 12 postoperation, the pigs were sacrificed in groups. To examine the regenerated bladder under the light microscope, retrieved tissue specimens were dissected and fixed in a 10% buffered formalin solution overnight. Samples were then dehydrated in graded alcohols and embedded in 4% paraffin. Tissue sections with a thickness of 3–5 µm were then prepared. Hematoxylin and eosin staining, Masson's trichrome, and immunohistochemistry were carried out to examine the morphology of the bladder regeneration *in vivo*.

In vivo angiogenesis

Tissue sections were stained with a CD₃₁ (antibody at a 1:50 dilution) and hematoxylin and eosin (H&E). To evaluate the microvascular density of the samples, the number of capillary vessels was counted in 10 randomly selected fields at 200× magnification.

Morphometric assessment of the blood vessels was carried out using the Lc index. The length density, Lc, is equal to the major or long axis to the minor or wide axis of each vessel.^{11,12} The difference between the Lc values in different fields represents the diversity in capillary morphology.

FIG. 1. The transplantation of bladder acellular matrix allografts (BAMA). The figure revealed the bladder replacement process by BAMA (5 × 5 cm), namely, the BAMA at time point 0. Color images available online at www.liebertpub.com/tea

Histological evaluation of the regenerated bladder

The histological structure of bladders was examined using H&E and Masson's trichrome staining. Collagen degradation and smooth muscle regeneration were evaluated by Masson's trichrome staining and anti-smooth muscle actin (anti-SMA) (1:100 dilution) immunohistochemistry. To evaluate the percentage of SMA- and collagen-positive areas, six random areas in each section were counted at 200 $\times$ magnification. Positive areas were then quantified using Image Pro[®] Plus, using the following equation: percent positive area = positive area/total tissue area.

The functional evaluation of isolated strips

Regenerated bladders were evaluated 12 weeks after surgery.^{3,13} Specimens were cut into 1.5 $\times$ 0.5 cm strips and placed in 12 mL of K-H solution. The temperature of solution was maintained at 37°C throughout the experiment with constant bubbling with 95% O₂ and 5% CO₂. The preload was set at 1.0 g and then equilibrated for at least 30 min before the testing of drugs. During drug testing, epinephrine was used at increasing concentrations from 10⁻⁸ to 10⁻⁴ mol/L to examine contractile effects, while potassium chloride was used at concentrations of 10⁻⁷ to 10⁻³ mol/L. Atropine was used at concentrations of

3 $\times$ 10⁻⁹, 1 $\times$ 10⁻⁸, and 1 $\times$ 10⁻⁷ mol/L to examine its inhibitory effect on epinephrine-induced contraction.

Statistical analysis

Results are expressed as the mean $\pm$ SD. Statistical analysis was evaluated using SPSS software. The unpaired Student's *t*-test was performed for comparison between the two groups, and *p*-values less than 0.05 were considered statistically significant.

Ethics statement

All experimental procedures were conducted in conformity with institutional guidelines for the Care and Use of Laboratory Animals in Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China and conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1996).

Results

In vivo angiogenesis

The microvessel density (MVD) of vessels at weeks 1, 2, 4, and 12 was revealed by H&E staining and CD₃₁ staining.

FIG. 2. CD₃₁ staining in 1, 2, 4, and 12 weeks. The figure revealed the neovascularization conditions at weeks 1, 2, 4, and 12 in the control (A–D) and experimental groups (E–H) by CD₃₁ staining (200 $\times$ magnification). The arrows indicated the CD₃₁ staining-positive structures. As the figure showed, with the passage of time, the number of blood vessels was on a rising trend, and the microvessel density (MVD) in experimental groups was much higher than control groups at the same time point. Statistical analysis of the MVD (I) and length density (J) revealed a significant difference in neovascularization between groups ($p < 0.01$), but no difference in length density ($p > 0.05$). Color images available online at www.liebertpub.com/tea

At each time point, the MVD in the experimental group was significantly higher than the control group ($p < 0.01$). The MVD at weeks 1, 2, 4, and 12 was 2.31 ± 1.29 , 5.15 ± 1.64 , 9.31 ± 2.42 , and 12.96 ± 2.67 in the experimental group and 0.46 ± 0.63 , 1.41 ± 1.25 , 4.73 ± 1.36 , and 7.02 ± 1.65 in the control group, respectively.

However, when vascular morphological differences were evaluated, there were no significant differences between the experimental and control groups ($p > 0.05$). The Lc evaluation index was 1.238 ± 0.103 , 1.652 ± 0.101 , 1.965 ± 0.102 , and 1.840 ± 0.803 in the experimental group and 1.248 ± 0.102 , 1.664 ± 0.106 , 1.929 ± 0.084 , and 1.873 ± 0.855 in the control group at weeks 1, 2, 4, and 12, respectively (Fig. 2).

The contracture rate of regenerated bladder

At 4 and 12 weeks, contracture of the bladder appeared in both groups. The experimental group showed significantly larger areas of regenerated bladder than the control group, and the contracture rate was significant lower. The contracture rates of the experimental group at 4 and 12 weeks were $19.16\% \pm 1.656\%$ and $24.08\% \pm 1.404\%$, while rates were $32.31\% \pm 1.23\%$ and $56.03\% \pm 2.919\%$ in the control group ($p < 0.01$), respectively (Fig. 3).

Histological evaluation of regenerated bladder

Compared with control, the BAMA showed more rapid degradation and cellularization in the experimental group, as well as a more organized arrangement of the epithelium. Next, Masson's staining and α -SMA staining were used to evaluate the percentage of fibers positive for collagen and muscle actin. The percentage of collagen-positive areas at 1, 2, 4, and 12 weeks was 79.30 ± 8.676 , 69.38 ± 6.745 , 55.43 ± 7.315 , and 36.29 ± 5.943 in the control group and 77.36 ± 9.716 , 67.84 ± 6.586 , 52.83 ± 7.249 , and 27.20 ± 6.607 in the experimental group, respectively. The differences at 4 and 12 weeks were statistically significant ($p < 0.05$). When α -SMA was examined, unexpected normal muscle fibers were observed in both groups at 1 and 2 weeks. However, at 4 and 12 weeks, significant differences were seen between the 2 groups (23.78 ± 4.498 compared with 14.05 ± 4.352 at 4 weeks and 45.83 ± 6.130 vs. 22.47 ± 4.515 at 12 weeks in the experimental and control groups, respectively; $p < 0.01$). The differences in collagen degradation and muscle growth indicate better regeneration in the experimental group (Figs. 4–6).

FIG. 3. Contracture conditions in 4 and 12 weeks. The figure revealed the contracture conditions in BAMA-regenerated bladder. The BAMA-regenerated bladder area was circled out by the dotted line. The BAMA-regenerated bladder area at 4 weeks in the experimental (A) and control groups (B). The BAMA-regenerated bladder area at 12 weeks in the experimental (C) and control groups (D). As the figure showed, the areas of BAMA-regenerated bladder in experimental groups were much bigger than control groups at the same time point. Statistical analysis of the contracture rate at 4 and 12 weeks in both groups; there was a significant difference between the two groups at both time points ($p < 0.01$) (E). Color images available online at www.liebertpub.com/tea

FIG. 4. H&E staining in 1, 2, 4, and 12 weeks. The figure revealed the histology morphological characteristics of the regenerated bladder at weeks 1, 2, 4, and 12 in the control (A–D) and experimental groups (E–H) at weeks 1, 2, 4, and 12 by hematoxylin and eosin staining (200× magnification). H&E, hematoxylin and eosin. Color images available online at www.liebertpub.com/tea

FIG. 5. Masson's trichrome in 1, 2, 4, and 12 weeks. The figure revealed the percentage of collagen-positive areas of regenerated bladder at weeks 1, 2, 4, and 12 in the control (A–D) and experimental groups (E–H) by Masson's trichrome (200× magnification). The arrows indicated the Masson's trichrome-positive structures. As the figure showed, the percentages of fibers positive for collagen in experimental groups were very nearly the same with control groups at weeks 1 and 2. While at weeks 4 and 12, the percentages of fibers positive for collagen in experimental groups were much lower than control groups. Statistical analysis of the percentage of collagen-positive areas at each time point (I). Data showed no significant differences between groups at 1 and 2 weeks ($p > 0.05$), while at 4 and 12 weeks, the differences between the control and experimental groups were statistically significant ($p < 0.05$). Color images available online at www.liebertpub.com/tea

FIG. 6. α -SMA staining in 4 and 12 weeks. The figure revealed the smooth muscle regenerated condition at weeks 4 and 12 in the control (A, B) and experimental groups (C, D) by α -SMA staining (200 $\times$ magnification). The arrows indicated the α -SMA staining-positive structures. As the figure showed, the smooth muscle regenerated condition in experimental groups was much better than control groups at weeks 4 and 12; statistical analysis of the percentage of α -SMA-positive areas (E) also revealed significant differences between the experimental and control groups at 4 and 12 weeks ($p < 0.05$). SMA, smooth muscle actin. Color images available online at www.liebertpub.com/tea

The evaluation of isolated strips in vitro

At 12 weeks, functional analysis of the regenerated bladders was carried out using isolated strips. The whole bladders consisted of BAMA-regenerated bladder and autogenous bladder, taking BAMA-regenerated bladder as the experimental group and autologous bladder as the control group. Bladders in both groups were stimulated with equal doses of potassium chloride, epinephrine, and atropine. With the increasing concentration of stimulants, the strips reached a

final contraction or diastolic reaction at the final concentration. In the VEGF-NP groups, potassium chloride got the final contracture rates $193.50\% \pm 8.74\%$ in experimental groups and $195.42\% \pm 11.62\%$ in control groups; epinephrine got $126.66\% \pm 6.11\%$ final contracture rates in experimental groups and $129.83\% \pm 6.89\%$ in control groups; and as for atropine, the final diastolic rates were $24.75\% \pm 4.07\%$ in experimental groups and $26.67\% \pm 4.20\%$ in control groups. There were no statistical differences between control groups and experimental groups ($p > 0.05$) in respect of diastolic

FIG. 7. The evaluation of isolated strips function. The figure revealed the diastolic contraction reaction of isolated strips stimulated by drugs. The contraction reaction stimulated by increasing concentrations of contraction stimulants in BAMA-regenerated bladder strips (A) and autogenous bladder strips (B), and the diastolic reaction stimulated by increasing concentrations of diastolic stimulants in BAMA-regenerated bladder strips (C) and autogenous bladder strips (D). As the figure showed, with the increasing concentration of stimulants, the more severe contraction or diastole reaction was observed, and statistical analysis of the final diastolic and contraction rate indicated that there was no significant difference between the two parts of the bladder ($p > 0.05$). Color images available online at www.liebertpub.com/tea

rates, and the same as contraction rates. The same conclusion was also applicable to the NO-VEGF-NP groups. In the NO-VEGF-NP groups, potassium chloride got the final contracture rates $188.46\% \pm 5.32\%$ in experimental groups and $185.02\% \pm 9.86\%$ in control groups; epinephrine got $133.29\% \pm 8.92\%$ in experimental groups and $135.02\% \pm 6.74\%$ in control groups; with regard to the final diastolic rates, atropine reached it at $20.97\% \pm 6.68\%$ in experimental groups and $22.37\% \pm 7.44\%$ in control groups. Statistical analysis indicated that there were also no significant differences in diastolic and contraction reaction between the two groups ($p > 0.05$). This test revealed that there were no significant differences between BAMA-regenerated (either VEGF-NPs added or not) bladder and autogenous bladder at 12 weeks (Fig. 7).

Discussion

BAMA has been used as an alternative biomaterial for bladder replacement in animal models.^{3,14} However, the onset of contracture *in vivo* during regeneration has always been a critical issue. After migrating from normal tissue to the collagen matrix tablet, cells have to assimilate nutrients and eliminate metabolites. For cells $\geq 3 \text{ mm}^3$, intracellular dispersion was not sufficient to support the survival of migrated cells,¹⁵ and so, blood vessels were the key to maintaining metabolic pathways. The induction of rapid angiogenesis after the implantation of BAMA is therefore of great significance for the regeneration of the whole tissue, and an insufficient physiological process to produce new sprouting vessels is considered to be a possible cause of graft contracture.^{4,5} Associating growth factor to BAMA therefore became a focus of research. In two early studies, Kanematsu *et al.* and Youssif *et al.* attempted to incorporate growth factor into BAMA, to improve the self-regeneration ability of the bladder.^{5,9} However, the results were disappointing; although the ability of the bladder to regenerate was improved for 2–3 weeks, the inhibition of contracture became insignificant over time due to loss of VEGF. We believe that lack of revascularization affects the entire regeneration process and that sustained long-term release of growth factors to promote angiogenesis may inhibit contracture.

VEGF-loaded PLGA NPs were prepared in 2010 in our laboratories¹⁰ and were embedded in the thermoresponsive hydrogel, Pluronic F127, in the BAMA. It was previously confirmed that the 2-month overall release rate of these NPs is $\sim 60\%$ and that the sustained release can last for 3 months.^{16,17} In addition, we verified that the release of VEGF *in vitro* promotes the proliferation of endothelial cells.¹⁰ Our previous research in rabbits witnessed that the contracture rate was significantly restrained when using VEGF-NP-modified BAMA ($2 \times 3 \text{ cm}$) to replace the bladder. On the basis of our research in rabbits, we took further observation in pig and used a bigger BAMA ($5 \times 5 \text{ cm}$) with modified VEGF-loaded NPs to substitute a partial bladder ($35\text{--}50\%$), to investigate the effects on bladder contracture.

With sustained stimulation from VEGF, the MVD in the experimental group was significantly higher than control ($p < 0.01$). There were no significant differences between the two groups with respect to the morphological characteristics of the microvessels ($p > 0.05$). This indicated the occurrence of angiogenesis during the whole process of bladder regeneration. Bladders in both groups had comparable vascular permeability and erythrocyte spillover. Although this may be because

VEGF was an efficient stimulator of angiogenesis,^{18–20} there was no significant effect on blood vessel maturation. In contrast, previous studies revealed that bFGF could greatly improve the quality of regenerated blood vessels.^{21,22} A possible next study would therefore be to combine bFGF and VEGF into the BAMA and test their combined effects on the bladder regeneration process.^{23,24} Furthermore, we also assessed the recovery of bladder function by examining the regeneration of bladder smooth muscle and degradation of the collagen matrix. We found that the percentage of α -SMA-positive area in the experimental group was higher than that in the control group ($p < 0.05$) at both 4 and 12 weeks, and the percentage of the collagen matrix-positive area of the experimental group was lower than the control group ($p < 0.05$). This data suggested that long-term sustained release of VEGF was not only capable of promoting muscle growth but also facilitating collagen matrix degradation, all of which benefit the reconstruction of bladder function.

To evaluate contracture of the acellular matrix, the most intuitive area of the regenerated bladder was used as the evaluation index and was then compared to the area of regenerated bladder at 4 and 12 weeks. Comparing the area change index with the bladder volume could therefore more objectively and accurately reflect the contracture conditions of the BAMA. At both 4 and 12 weeks, the contracture rate in the experimental group was much lower than the control group.

H&E staining was carried out in both groups, and there was no significant difference in morphology and structure between the regenerated and host bladders. By evaluating the function of isolated host and BAMA-regenerated bladder strips *in vitro*, we found that there was no significant change in function in either group. Importantly, the addition of NPs did not result in an abnormal bladder.

In short, this is the first nanomedicine approach for sustained release of VEGF to successfully inhibit the contracture of regenerated bladders after BAMA implantation. We believe that this will be a novel and effective method to resolve the problem of contracture in the field of tissue engineering. Potential issues with contracture and a long-term drug delivery system are realized, and further optimization studies will be required. It is important to note that a few limitations are associated with the present study. One of the examples is that some small contractures still existed. We presume that this might be caused by the following reasons: (1) the quantity of embedded VEGF was not sufficient; (2) results of immunohistochemical analysis showed that there was no difference in the vascular morphology between the experimental and control groups. Both groups had signs of overflow of red blood cells as well as irregular blood vessels. Since bFGF could greatly improve the quality of regenerated blood vessels,^{18,19} we propose that bFGF and VEGF should be combined into BAMA and their effects on bladder regeneration be monitored; (3) during regeneration, there was significant collagen deposition, which had a negative impact on the normal function of VEGF, suggesting that collagen degradation should be taken into consideration when promoting regeneration.²⁵ Further studies are warranted to address these factors.

Acknowledgments

The authors would like to acknowledge the helpful advice and comments of Dr. Yuandong Peng from Baylor

College of Medicine. This work was supported by the National Nature Science Foundation (Grant No. 81170639, 2012–2015); the Natural Science Foundation of Shanghai Municipal Health Bureau (No. 20114Y079); and the Medicine and Engineering Foundation of Shanghai Jiaotong University (No. YG2011MS33).

Disclosure Statement

None of the authors has any conflict of interest to disclose.

References

- Schmitz, M., Bertram, M., Kneser, U., Keller, A.K., and Horsch, R.E. Experimental total wrapping of breast implants with acellular dermal matrix: a preventive tool against capsular contracture in breast surgery. *J Plast Reconstr Aesthet Surg* **66**, 1382, 2013.
- Nayyer, L., Patel, K.H., Esmaili, A., Rippel, R.A., Birchall, M., *et al.* Tissue engineering: revolution and challenge in auricular cartilage reconstruction. *Plast Reconstr Surg* **129**, 1123, 2012.
- Brown, A.L., Farhat, W., Merguerian, P.A., Wilson, G.J., Khoury, A.E., *et al.* 22 week assessment of bladder acellular matrix as a bladder augmentation material in a porcine model. *Biomaterials* **23**, 2179, 2002.
- Cassell, O., Stefan Hofer, O.P., Morrison, W.A., and Knight, K.R. Vascularisation of tissue-engineered grafts: the regulation of angiogenesis in reconstructive surgery and in disease states. *Br J Plast Surg* **55**, 603, 2002.
- Youssef, M., Shiina, H., Urakami, S., Gleason, C., Nunes, L., *et al.* Effect of vascular endothelial growth factor on regeneration of bladder acellular matrix graft: histologic and functional evaluation. *Urology* **66**, 201, 2005.
- Davies, N.H., Schmidt, C., Bezuidenhout, D., and Zilla, P. Sustaining neovascularization of a scaffold through staged release of vascular endothelial growth factor-A and platelet-derived growth factor-BB. *Tissue Eng Part A* **18**, 26, 2011.
- Schmidt, C., Bezuidenhout, D., Higham, L., Zilla, P., and Davies, N.H. Induced chronic hypoxia negates the pro-angiogenic effect of surface immobilized heparin in a polyurethane porous scaffold. *J Biomed Mater Res Part A* **98**, 621, 2011.
- Loai, Y., Yeager, H., Coz, C., Antoon, R., Islam, S.S., *et al.* Bladder tissue engineering: tissue regeneration and neovascularization of HA-VEGF-incorporated bladder acellular constructs in mouse and porcine animal models. *J Biomed Mater Res Part A* **94**, 1205, 2010.
- Kanematsu, A., Yamamoto, S., Ozeki, M., Noguchi, T., Kanatani, I., *et al.* Collagenous matrices as release carriers of exogenous growth factors. *Biomaterials* **25**, 4513, 2004.
- Geng, H., Song, H., Qi, J., and Cui, D. Sustained release of VEGF from PLGA nanoparticles embedded thermo-sensitive hydrogel in full-thickness porcine bladder acellular matrix. *Nanoscale Res Lett* **6**, 1, 2011.
- Safi, J., DiPaula, A.F., Riccioni, T., Kajstura, J., Ambrosio, G., *et al.* Adenovirus-mediated acidic fibroblast growth factor gene transfer induces angiogenesis in the non-ischemic rabbit heart. *Microvasc Res* **58**, 238, 1999.
- Zhu, W.D., Xu, Y.M., Feng, C., Fu, Q., Song, L.J., *et al.* Bladder reconstruction with adipose-derived stem cell-seeded bladder acellular matrix grafts improve morphology composition. *World J Urol* **28**, 493, 2010.
- Chen, B.S., Zhang, S.L., Hongquan, G., Pan, J., and Chen, F. *Ex vivo* functional evaluation of isolated strips in BAMG tissue-engineered bladders. *Int J Artif Organs* **32**, 159, 2009.
- Dahms, S.E., Piechota, H.J., Dahiya, R., Lue, T.F., and Tanagho, E.A. Composition and biomechanical properties of the bladder acellular matrix graft: comparative analysis in rat, pig and human. *Br J Urol* **82**, 411, 1998.
- Griffith, L.G., and Naughton, G. Tissue engineering—current challenges and expanding opportunities. *Science* **295**, 1009, 2002.
- Cui, D. Advances and prospects on biomolecules functionalized carbon nanotubes. *J Nanosci Nanotechnol* **7**, 4, 2007.
- Cui, D., Tian, F., Coyer, S.R., Wang, J., Pan, B., *et al.* Effects of antisense-Myc-conjugated single-walled carbon nanotubes on HL-60 cells. *J Nanosci Nanotechnol* **7**, 4, 2007.
- Yockman, J.W., Kastenmeier, A., Erickson, H.M., Brumbach, J.G., Whitten, M.G., *et al.* Novel polymer carriers and gene constructs for treatment of myocardial ischemia and infarction. *J Control Release* **132**, 260, 2008.
- Mataveli, F.D.A., Han, S.W., Nader, H.B., Mendes, A., Kanishiro, R., *et al.* Long-term effects for acute phase myocardial infarct VEGF165 gene transfer cardiac extracellular matrix remodeling. *Growth Factors* **27**, 22, 2009.
- Fujita, M., Ishihara, M., Simizu, M., Obara, K., Ishizuka, T., *et al.* Vascularization *in vivo* caused by the controlled release of fibroblast growth factor-2 from an injectable chitosan/non-anticoagulant heparin hydrogel. *Biomaterials* **25**, 699, 2004.
- Bruick, R.K., and McKnight, S.L. Building better vasculature. *Genes Dev* **15**, 2497, 2001.
- Henry, T.D., Annex, B.H., McKendall, G.R., Azrin, M.A., Lopez, J.J., *et al.* The VIVA trial vascular endothelial growth factor in ischemia for vascular angiogenesis. *Circulation* **107**, 1359, 2003.
- Ferrara, N. Role of vascular endothelial growth factor in the regulation of angiogenesis. *Kidney Int* **56**, 794, 1999.
- Nillesen, S., Geutjes, P.J., Wismans, R., Schalkwijk, J., Daamen, W.F., *et al.* Increased angiogenesis and blood vessel maturation in acellular collagen-heparin scaffolds containing both FGF2 and VEGF. *Biomaterials* **28**, 1123, 2007.
- Chen, X., and Li, Y. Role of matrix metalloproteinases in skeletal muscle: migration, differentiation, regeneration and fibrosis. *Cell Adh Migr* **3**, 337, 2009.

Address correspondence to:

Hongquan Geng, MD, PhD

Department of Pediatric Urology

Xinhua Hospital

Shanghai Jiao Tong University School of Medicine

1665 Kongjiang Road

Shanghai 200092

People's Republic of China

E-mail: ghongquan@hotmail.com

Fang Chen, MD, PhD

Department of Urology

Children's Hospital

Shanghai Jiao Tong University School of Medicine

1440 Beijing West Road

Shanghai 200002

People's Republic of China

E-mail: chenfang007@hotmail.com

Received: October 29, 2013

Accepted: June 13, 2014

Online Publication Date: July 24, 2014