BASIC SCIENCE

A Preliminary Study of Constructing the Tissue-Engineered Corpus Cavernosum With Autologous Adipose Stem Cells In Vivo

Zilong Cao, MD, Liqiang Liu, MD, Hu Jiao, MD, Cheng Gan, MD, Jia Tian, MD, Tiran Zhang, MD, and Bing Han, MD

ABSTRACT

Introduction: The autologous skin flap is still the mainstream method for penile reconstruction, but it is very difficult to reconstruct a functional corpus cavernosum. Tissue engineering provides a new idea aiming to restore the damaged or absent corpus cavernosum.

Aim: To assess the feasibility of constructing the tissue-engineered corpus cavernosum with autologous adipose stem cells in a rabbit model.

Methods: A total of 30 New Zealand male white rabbits. Among them, 20 rabbits were used to obtain the original corpus cavernosum which were used to prepare the acellular corporal scaffolds (ACSs). The others were used for acquiring autologous adipose stem cells (ADSCs) and constructing tissue-engineered corpus cavernosum in vivo.

Outcome: ACSs were obtained from rabbit penile tissues through an established decellularization procedure. Rabbit autologous ADSCs as seed cells were harvested and expanded. The ADSCs seeded and unseeded ACSs were implanted back into the intramuscular and subcutaneous site in vivo, and the tissue-engineered corpus cavernosum was harvested and analyzed with gross morphology, histological staining, and real-time PCR assay after 1, 3, and 6 months.

Results: ACSs were successfully prepared. The cell non-cytotoxicity and integrity of micro-architecture of ACSs was confirmed in vitro. The cell-seeded scaffold in the intramuscular group was considered as the better strategy for constructing the tissue-engineered corpus cavernosum compared with the other groups. Some α-SMA and CD31 positive cells were detected and identified by immunofluorescent staining and real-time PCR assay in the tissue-engineered corpus cavernosum.

Clinical Translation: This study provides a new method for constructing the tissue-engineered corpus cavernosum.

Strengths and Limitations: First, it is urgent to improve the transformation rate of the endothelial cells and smooth muscle cells from ADSCs. Second, the scaffold harvested in this study was not a complete matrix. Third, further study is needed to explore the potential mechanism of which scaffolds are more suitable for living in intramuscular rather than subcutaneous environment.

Conclusion: In this study, we used the autologous ADSCs as seed cells, the acellular corpus cavernosum as scaffolds, and implanted the grafts back into the rabbit model to preliminarily construct the tissue-engineered corpus cavernosum. This study would provide help for further development in tissue-engineered corpus cavernosum. **Cao Z, Liu L, Jiao H, et al. A Preliminary Study of Constructing the Tissue-Engineered Corpus Cavernosum With Autologous Adipose Stem Cells In Vivo. Sex Med 2022;10:100563.**

Key Words: Tissue engineering; Corpus cavernosum; Acellular corporal scaffolds; Adipose stem cells

INTRODUCTION

Penile defects are commonly seen in various congenital and acquired diseases, such as penis dysplasia, trauma, and tumor resection.[1] Phalloplasty with autologous skin flap is still the mainstream method to solve such problems. Although the neophallus can obtain certain appearance, it lacks normal corpus

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Plastic Surgery Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

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cavernous structure. As a result, it cannot achieve sufficient hardness to complete sexual intercourse, so a prosthesis or autologous bone must be implanted.[2] Prosthesis has a high risk of infection, rejection, exposure, and mechanical failure. The autologous bone deforms and absorbs easily.[3-5] In addition, it is impossible to simulate the normal erection process. Similarly, allotransplantation is an optional treatment; however, it is limited by ethics, lifelong use of immunosuppressants, host diseases and rejection.[2,6] Therefore, there is an urgent need to construct a functional corpus cavernosum.

The tissue structure of corpus cavernosum is complex, with sinusoidal tissue formed by vascular endothelial cells and smooth muscle cells inside, and dense tunica albuginea outside.[6,7] The development of tissue engineering and regenerative medicine can offer new hope to potentially restore the normal function of the corpus cavernosum.[6,8] For a tissue engineered corpus cavernosum to be successful, an appropriate acellular scaffold and suitable seed cells must be used.[9]

In the past studies, smooth muscle cells, vascular endothelial cells, and chondrocytes have been used as seed cells in tissue-engineered corpus cavernosum.[10-12] However, as adult cells, they only have the ability of proliferation without differentiation,[9] which cannot achieve the normal cell composition and distribution in the corpus cavernosum. ADSCs are easy to harvest and will not cause great damage to donors. As adult stem cells, they have the capacity for long-term self-renewal and differentiation into other adult cells.[13,14] Studies have confirmed that ADSCs have the characteristics of differentiation into vascular endothelial cells and smooth muscle cells.[15-17] Furthermore, the quantity of stem cells in adipose tissue is more than bone marrow and peripheral blood.[14] Therefore, ADSCs are potentially good seed cells for tissue-engineered corpus cavernosum.

Scaffolds for tissue engineering can be divided into polymers and biomaterial scaffolds. Polymers such as polycaprolactone, polyglycolic acid, hydrogel scaffolds, possess angiogenic and degradable properties.[18-20] However, they are quite different from the micro-architecture of the corpus cavernosum, so they cannot simulate the normal physiological functions. A biomaterial scaffold is regarded as a natural tissue that retains the original specific extracellular matrix structure without cell composition. It provides an ecological niche for the seeded cells.[21,22] Therefore, ACS maybe a more suitable scaffold material.

The objective of this study was to assess the feasibility of constructing the tissue-engineered corpus cavernosum with autologous adipose stem cells in an in vivo rabbit model.

MATERIALS AND METHODS

Study Design

A total of 30 New Zealand male white rabbits, aged 6 months and weighing between 3.5 and 4.5 kg, were selected. Among them, 20 rabbits were selected as ACSs acquisition group to obtain the original corpus cavernosum which were used to prepare the ACSs. The other 10 were selected as ADSCs acquisition-scaffold transplantation group for acquiring autologous ADSCs and constructing tissue-engineered corpus cavernosum in vivo. The animal experimental protocol was approved by the Animal Ethics Committee of the Plastic Surgery Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College.

Preparation of Rabbit ADSCs

After general anesthesia, a 5 cm longitudinal incision was performed on the posterior neck of the rabbits, and the adipose tissue was dissected and harvested. The ADSCs were obtained according to routine procedures.[17,23] Briefly, the adipose tissue was washed with phosphate buffered saline (PBS) to remove the red blood cells; then, the visible blood vessels, muscles and fascia were removed with sterile scissors. Subsequently, the adipose tissue was cut into small particles and digested using twice the volume of 0.1% collagenase type I at 37°C 100RPM/min for 50 minutes. The remaining tissue was filtered, centrifuged, resuspended and cultured in low glucose complete medium (Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin). The medium was changed every 3 days under standard incubation conditions (37°C, 5% CO_2). The cells were expanded to the third passage when the quantity of cells was sufficient for reseeding on the ACSs.

Multi-Differentiation Potential of Rabbit ADSCs

Adipogenic and osteogenic differentiation: the third passage ADSCs were inoculated into 6 well plates at the density of $2*10^4$ /mL. When they reached 70% confluency, the cells were cultured with adipogenic or osteogenic induction medium (Oricell), which was changed every 3 days for 21 days. The differentiated cells were identified by oil red O staining and alizarin red staining, respectively.

Chondrogenic differentiation: the third passage ADSCs were transferred into a 15ml centrifuge tube at the density of $4*10^5$ /mL. After centrifuging, the cells were cultured with 0.5 ml chondrogenic induction medium (Oricell), which was changed every 3 days for 21 days. The differentiated cells were identified by alcian blue staining.

Preparation of ACSs

The ACSs were obtained according to routine procedures.[8,9] Briefly, the New Zealand male white rabbits were executed with aeroembolism through the ear vein, and the penile tissue was cut off at the lower edge of the pubis under sterile conditions. The corpus cavernosum was dissected along the surface of the tunica albuginea, and the urethra on the ventral side and other redundant tissues were removed. The entire corpus cavernosum was cut into 5 mm thickness sections. Subsequently, the corporal

scaffolds were washed with acellular fluid (1% TritonX-100 and 0.1% ammonium hydroxide in distilled water) for 21 days at 4 °C and 100 RPM/min, to remove the cellular debris. Finally, the corporal scaffolds were washed with PBS for 24 hours, to rinse the residual acellular fluid. Hematoxylin-eosin (HE) staining, Masson trichrome staining and 4′,6-diamidino-2-phenylindole (DAPI) staining were randomly performed at different time periods of 0, 7, 14, and 21 days, respectively.

In order to test the cytotoxicity of ACSs, the extract solutions from the ACSs were used in a Cell Counting Kit-8 (CCK-8) assay. Briefly, the prepared ACSs were soaked in the complete medium for 48 hours to prepare the extract solutions, with the gradient concentration (mg (ACSs) /mL (medium)) of 100 mg/mL, 50 mg/mL, 25 mg/mL and 0 mg/mL, respectively. A total of 100 μL of the third passage ADSCs suspension was inoculated into 96 well plates at a density of $5*10^4$ /mL. The extract solutions were changed after 24 hours of cell adhesion. After 24, 48 and 72 hours of culture, the solutions were replaced with 10μL CCK-8 solution and 90 uL complete medium. The optical density (OD) values at 450 nm were measured with a microplate reader after 2 hours of culture.

Seeding and Proliferation of ADSCs in ACSs In Vitro

The third passage ADSCs were digested, centrifuged and resuspended at a concentration of $20*10^6$ /mL. After soaking in a complete medium for 24 hours, the ACSs were transferred to a 48 well plate, and each scaffold was placed in an individual well. The cell suspension was injected into the ACSs repeatedly at different sites using a 22 gauge needle. The cell-seeded ACSs were incubated for 12 hours to allow the cells to attach to the ACSs. In addition, cell unseeded ACSs were set as the control group.

The cell seeded ACSs were transferred to a 24 well plate, and incubated with low glucose complete medium, which was changed every 3 days for 28 days. HE staining and DAPI staining were randomly performed to observe the integrity of microarchitecture and morphology of cells. Furthermore, a 5-Ethynyl-2′-deoxyuridine (EdU) apollo 567 in vitro kit (Solarbio) was used to assess the proliferation of seeded cells. Briefly, 1 mL of prepared EdU solution was added to each well. After incubating for 2 hours, the cell-seeded scaffold was fixed with 4% paraformaldehyde for 24 hours, and embedded in paraffin. After dewaxing, the sections were processed by Apollo and Hoechst 33342, respectively, then observed by fluorescence microscope.

Implantation and Retrieval of Cell Seeded and Unseeded ACSs In Vivo

As noted, after incubating for 12 hours in the 48 well plate, the autologous ADSCs seeded and unseeded ACSs were immediately implanted back into the rabbits from which the primary adipose tissues were obtained. Based on whether the ADSCs were seeded and the implanted position of the ACSs, 4 different groups were determined: cell-seeded scaffold in the intramuscular (SSI) group and the subcutaneous (SSS) group, cell-unseeded scaffold in the intramuscular (u-SSI) group and the subcutaneous (u-SSS) group. Briefly, an incision was cut on the inner side of both proximal thighs to expose adductor muscles; then the sarcolemma was dissected, and the cell-seeded and unseeded ACSs were implanted on both sides, respectively. The sarcolemma and skin were sutured with 5−0 absorbable suture. Similarly, an incision was cut on both sides of the back to expose the subcutaneous layer. The seeded and unseeded ACSs were implanted on both sides. The skin was sutured with 5−0 absorbable suture.

The implanted tissue-engineered corpus cavernosum were retrieved 1 month, 3 months and 6 months, respectively. The local responses of the rabbits were assessed; gross morphology was used to evaluate the appearance of ACSs; HE staining was used to observe the micro-architecture of scaffolds and the infiltration of surrounding inflammatory cells. Based on the above results, the optimal of the 4 groups was determined preliminarily.

Next, immunofluorescent staining was used to detect CD31 and α-SMA in the optimal group. Briefly, after routinely dewaxing, antigen repairing and blocking, the sections were incubated with primary antibodies against CD31 (1:25, mouse-anti-rabbit, Novus) and α-SMA (1:1000, mouse-anti-rabbit, Novus) overnight at 4°C. Subsequently, the sections were washed and incubated with a secondary antibody (1:500, Dlight 488 Goat Anti-mouse IgG (H+L), BOSTER) for 1 hour at room temperature. Lastly, the sections were washed and mounted with an antifade mounting medium with DAPI. Immuno-stained samples were imaged by fluorescence microscope.

In addition, Real-Time PCR was used for quantitative analysis of CD31 and α-SMA in the optimal group. Briefly, the total RNA was extracted from cell seeded ACSs using Trizol according to the manufacturer's instructions. The concentration of RNA was measured with NanoDrop spectrophotometer. cDNA was synthesized through reverse transcription using the FastKing-RT SuperMix (Tiangen). Primer sequences for amplification of CD31 and α-SMA were shown in Table 1. The real-time PCR assay was performed by SuperReal PreMix Plus (Tiangen). Relative mRNA expression was analyzed by the comparative cycle threshold $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

For all assays, statistics were performed by GraphPad Prism software (version 9.3.1). All results were expressed as mean value ± standard deviation (SD) from 3 independent experiments. One-way ANOVA was used for statistical analysis; $P < .05$ was considered statistically significant.

RESULTS

Morphology and Multipotential Differentiation of ADSCs

Primary ADSCs were successfully isolated from rabbit adipose tissue. After 48 hours of culture, ADSCs began to proliferate, and reached 80%−90% confluence 5−7 days later. After cell

Table 1. The primer sequences for amplification of CD31 and α-SMA

Gene	Primer Sequence
CD31	F: GTGATAATTGCCGCCTTGAT
	R: GTTGGGATCTGACACGGTCT
α-SMA	F: CTAGTTCTAGCTTGCGAGCGG
	R: CCAGCTGGTTCCATCCCAATA

passage, cells proliferated rapidly and reached 90% confluence after 48−72 hours. The third passage cells showed a uniform spindle-shape and were well-distributed. (Figure 1A)

After adipogenic induction, the morphology of ADSCs changed from spindle to polygonal shaped, and droplets appeared and aggregated in cells. Cells were stained red with oil red O staining (Figure 1B). After osteogenic induction, a large amount of extracellular matrix was secreted, and mineralized nodules were formed gradually, and the cells were positive for alizarin red staining (Figure 1C). After chondrogenic induction, the chondrogenic pellet increased gradually and became round, and the pellets were colored blue with alcian blue staining (Figure 1D).

Characteristics of ACSs

After 21 days of decellularization, the gross morphology of corporal scaffolds was unchanged; the color turned from red to white, and the texture changed to soft (Figure 2A-C). Tissue samples from different time periods (0, 7, 14 and 21 days,) were stained and observed under a microscope. HE staining showed that the micro-architecture of corporal scaffolds was intact, and the cellular and nuclear components gradually decreased, eventually without cell remnants in the visual field after 21 days (Figure 3A-D). Masson staining showed that the red muscle fibers gradually decreased, and the blue collagen fiber structure was not damaged (Figure 3E-H). DAPI staining showed that the blue stained nuclei gradually decreased and disappeared (Figure 3I-L).

The cytotoxicity of acellular scaffolds was tested by CCK8 assay. The extract solutions with different gradient concentrations were used to culture cells for 24 h, 48 h, and 72 h. The results of OD values showed that there were no significant differences ($P > .05$) in cell growth for each group at different time periods (Figure 4).

Construction of Tissue-Engineered Corpus Cavernosum In Vitro

The third passage ADSCs were seeded on the ACSs and cultured for 28 days in vitro. HE staining showed that the corporal

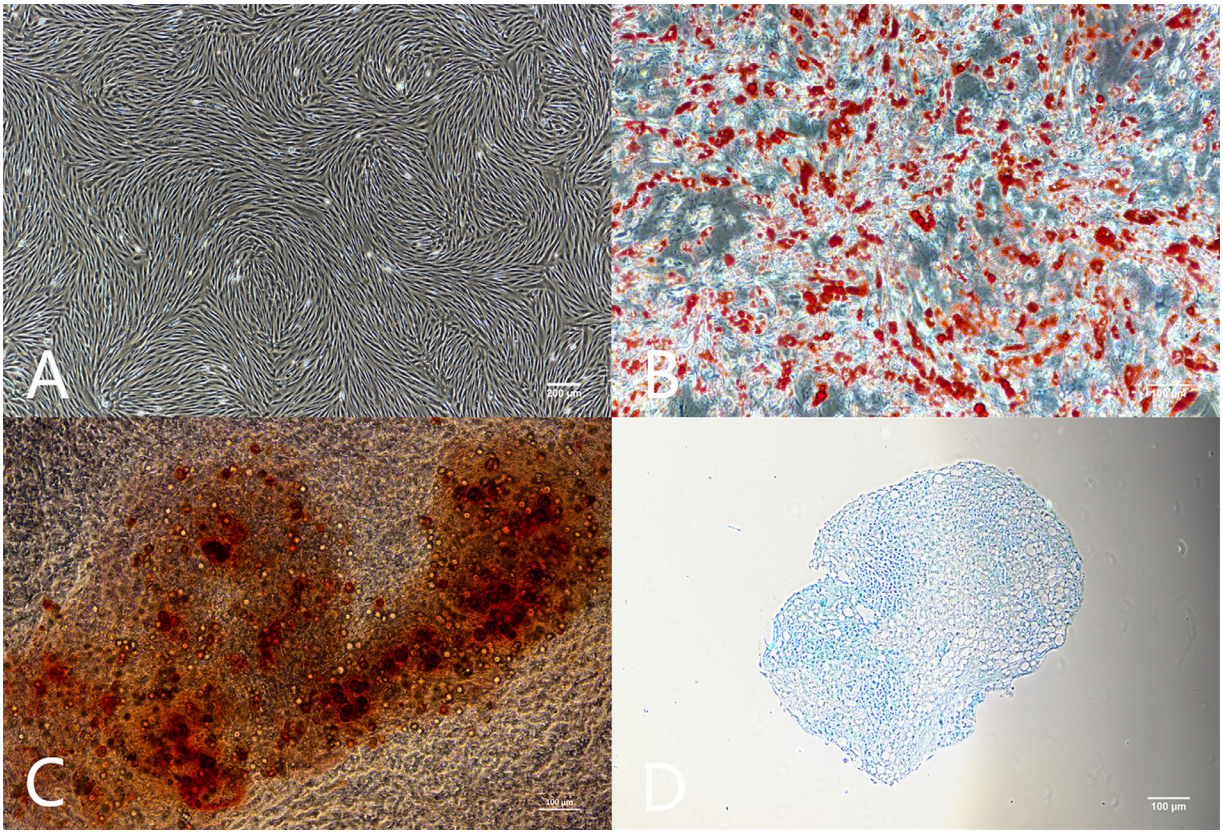

Figure 1. The morphology and multipotential differentiation of ADSCs. The third passage cells showed uniform spindle shaped and well-distributed (A). After adipogenic, osteogenetic and chondrogenic induction, the cells were positive for oil red O staining (B), alizarin red staining (C) and alcian blue staining (D), respectively.

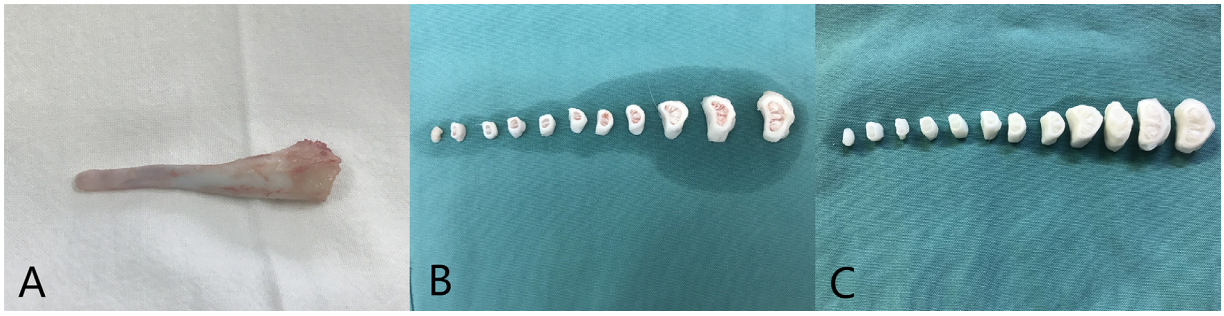

Figure 2. The gross morphology of corporal scaffolds during decellularization. (A) completed corporal scaffolds before decellularization. (B) sectional corporal scaffolds before decellularization. (C) sectional corporal scaffolds after 21 days of decellularization.

scaffolds maintained normal micro-architecture (Figure 5A). DAPI staining showed that the seeded cells were well-distributed in the tissue-engineered corpus cavernosum, but the cell density was lower than normal (Figure 5B). The unseeded ACSs showed no cell growth (Figure 5C-D). The proliferation of viable cells was assessed by EdU staining. The cells integrated with EdU showed red under a fluorescence microscope, which corresponded to DAPI staining, indicating the cells proliferated well in the ACSs, and the preliminary construction of tissue-engineered corpus cavernosum in vitro (Figure 5E-G).

Construction of Tissue-Engineered Corpus Cavernosum In Vivo

In the follow-up, 1, 3, and 6 months post-operation, no adverse complications were observed in all rabbits. All incisions healed well, with no redness and infection. The grafts from the SSS and u-SSS groups were easily dissected from the surrounding tissue; whereas, the grafts from the SSI and u-SSI groups grew closely with the surrounding tissue.

Gross morphology showed that all the SSI and u-SSI groups maintained a normal appearance without deformation and fibrous encapsulation. By contrast, all the SSS and u-SSS groups were completely squashed, and fibrosis occurred (Figure 6A-D).

HE staining showed that different degrees of inflammatory cell infiltration in all 4 groups within 1 month post-operation (Figure 7A). The inflammatory reaction gradually disappeared within 3 to 6 months post-operation. The SSI group maintained a normal micro-architecture, the collagen structure was still preserved, and abundant adipocytes and new blood vessels were seen inside the scaffold (Figure 7B-C). However, the normal micro-architecture of the other 3 groups disappeared, showing cell arrangement disorder and collagen deposition (Figure 7D-F).

Therefore, the SSI group was preliminarily considered as the better strategy to construct the tissue-engineered corpus cavernosum, compared with the other 3 groups. Next, immunofluorescent

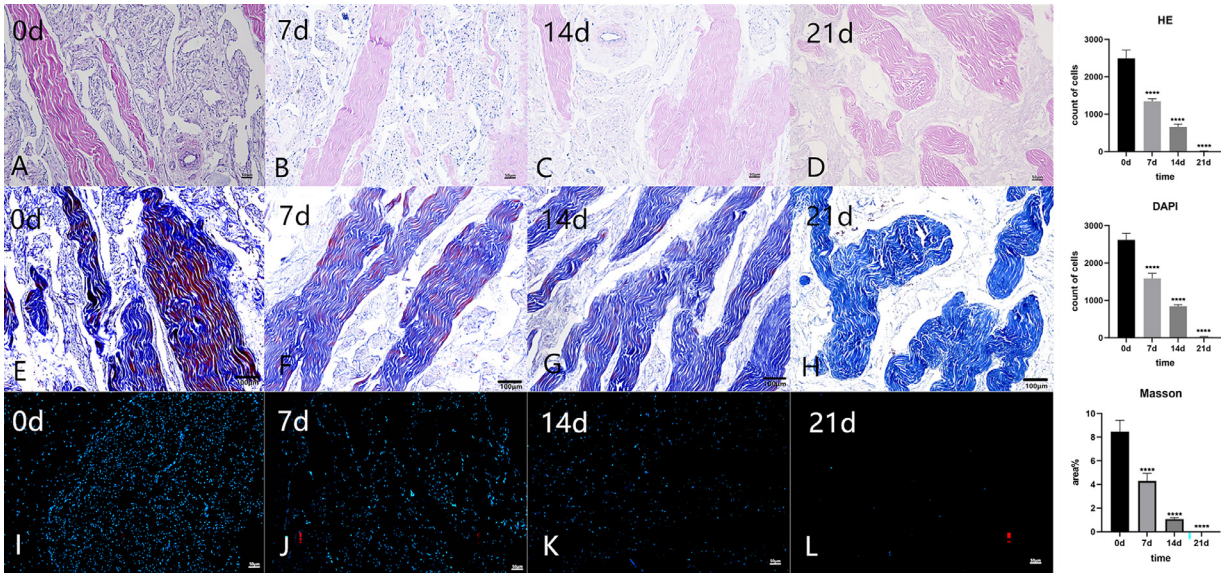

Figure 3. The histological staining of corporal scaffolds during decellularization. HE staining (A-D), Masson trichrome staining (E-H) and DAPI staining (I-L). The results showed that the micro-architecture and collagen fiber of corporal scaffolds was intact, the cellular components gradually decreased to disappeared with the extension of acellular time. **** means $P < .0001$.

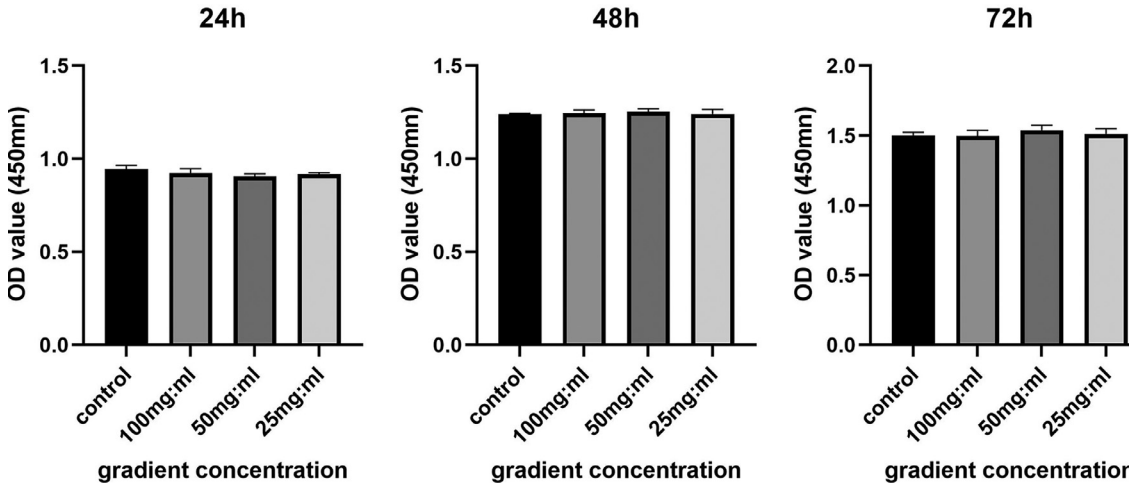

Figure 4. Cell Counting Kit-8 assay was performed to test the cytotoxicity of acellular scaffolds. After 24, 48, and 72 hours of culture, the results showed that there was no significant difference among the OD values of different groups ($P > .05$).

staining was performed to detect the differentiation of cells in the SSI group. The results revealed some CD31 and α-SMA positive cells in the scaffold at a different period, indicating the existence of smooth muscle cells and endothelial cells (Figure 8A-F). Subsequently, the real-time PCR assay was performed to detect the mRNA expression of CD31 and α-SMA in the SSI group. The results showed that CD31 and α-SMA mRNA were expressed in normal native tissue and tissue-engineered corpus cavernosum but not in ACSs. Furthermore, the quantitative expression of α- CD31 and α-SMA was gradually increased with the extension of implantation time (Figure 9).

DISCUSSION

In current treatment, the autologous skin flap is still the mainstream method for penile reconstruction, but the method used to reconstruct a functional corpus cavernosum is very difficult.[2] Tissue engineering provides a new idea aiming to restore the damaged or absent corpus cavernosum.[9] In order to successfully construct the tissue-engineered corpus cavernosum, seed cell, scaffold, as well as the micro-environment are the principal components. In addition, the immunogenicity, toxicity, host inflammatory response, and mechanical properties are the critical evaluation indexes.[18]

The cellular components of the corpus cavernosum are diverse, therefore the appropriate seed cell is urgently needed. As adult cells have no differentiation ability, stem cells are an alternative and have the capacity for long-term self-renewal and differentiation into different types of cells. In this study, in order to minimize the immunological rejection, the autologous ADSCs were successfully isolated as seed cells, and their characteristics of osteogenetic, adipogenic, and chondrogenic differentiation were revealed.

The appropriate scaffold could provide an excellent environment for seed cell attachment and proliferation.[24] During decellularization, seeking the balance between complete decellularization and structural preservation is very important.[21] After 21 days of decellularization, HE staining, Masson trichrome staining, and DAPI staining indicated that the scaffolds were without cell remnants and retained basic micro-architecture. In order to prove whether the scaffold is safe for cell growth, CCK8 assay was tested to indicate the scaffolds had no cytotoxicity; furthermore, the cell-seeded ACSs were cultured 28 days in vitro, HE staining, DAPI staining and EdU staining indicated the seed cells well distributed and proliferated in the scaffolds. Growth signals are important factors related to the differentiation of ADSCs.[25] Previous studies reported that some low levels of cytokines, such as VEGF, IGF-1, and TGF-β remained in the ACSs after decellularization,[6,8] which could promote the transformation of ADSCs into vascular endothelial and smooth muscle cells.[16,26] However, it is doubtful whether the remaining cytokines have bioactivity. We thought the scaffold should be considered as the niche for the seeded cells to attach, rather than inducing cell differentiation by the residual cytokines.

In order to determine an optimal micro-environment for constructing the tissue-engineered corpus cavernosum, the cell-seeded and unseeded ACSs were implanted into the intramuscular and subcutaneous site of the rabbits. After 1, 3, and 6 months of follow-up, the grafts were harvested. The results showed that the grafts had good compatibility with the surrounding tissue, and there were no complications in the recipient rabbits. According to the gross morphology, the SSS and u-SSS groups occurred deformation and fibrous encapsulation, compared with the SSI and u-SSI groups that maintained a normal appearance. Based on the HE staining, the SSI group maintained normal micro-

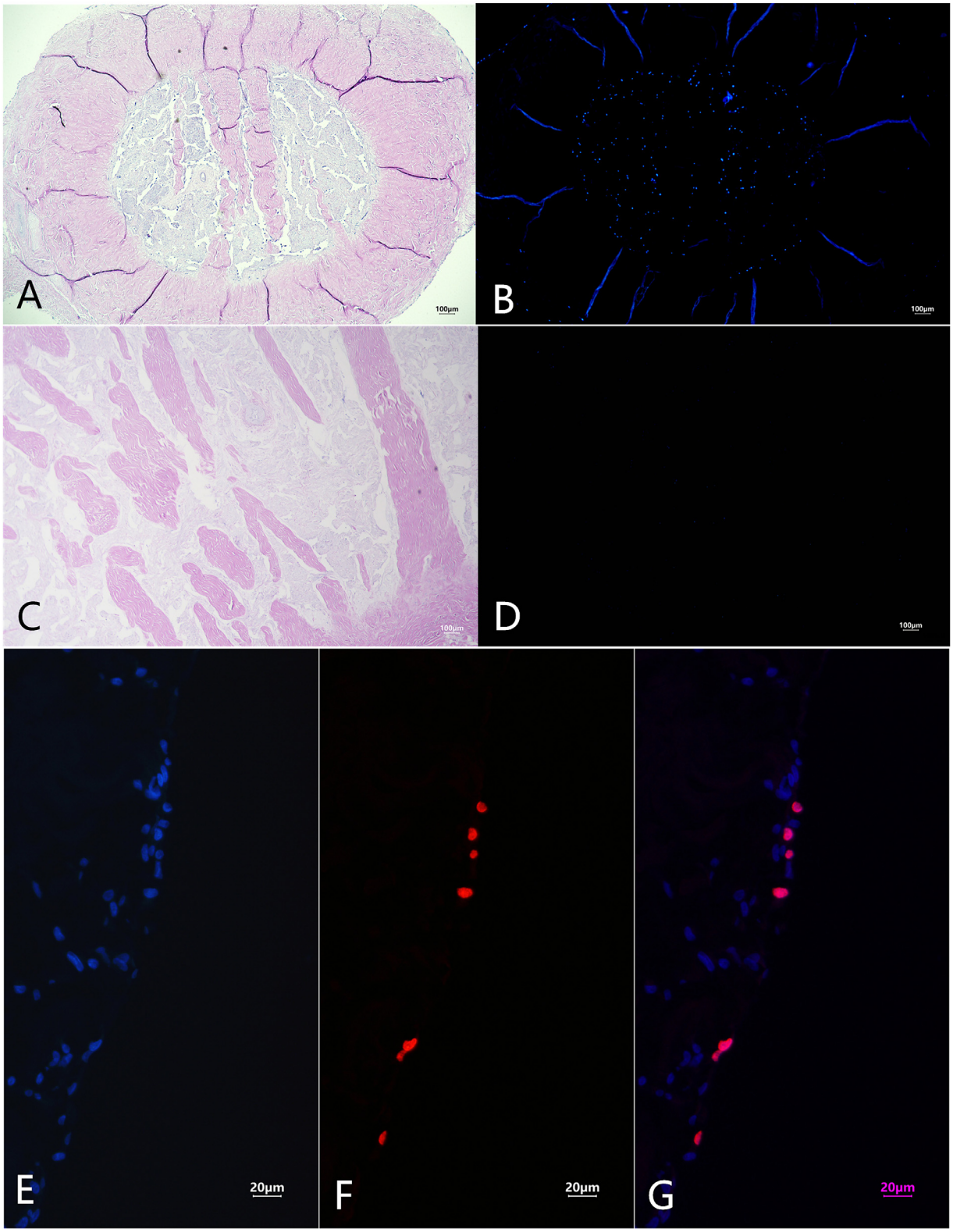

Figure 5. The histology of tissue-engineered corpus cavernosum and cell proliferation analysis after 28 days of culture in vitro. HE staining (A) and DAPI staining (B) showed that the tissue-engineered corpus cavernosum maintained normal micro-architecture, the seeded cells were well-distributed, but the cell density was lower than normal. However, the unseeded ACSs showed no cell growth (C and D). DAPI staining (E), EdU staining (F) and their merge (G) indicated that the cells proliferated well in the ACSs, and the preliminary construction of tissue-engineered corpus cavernosum in vitro.

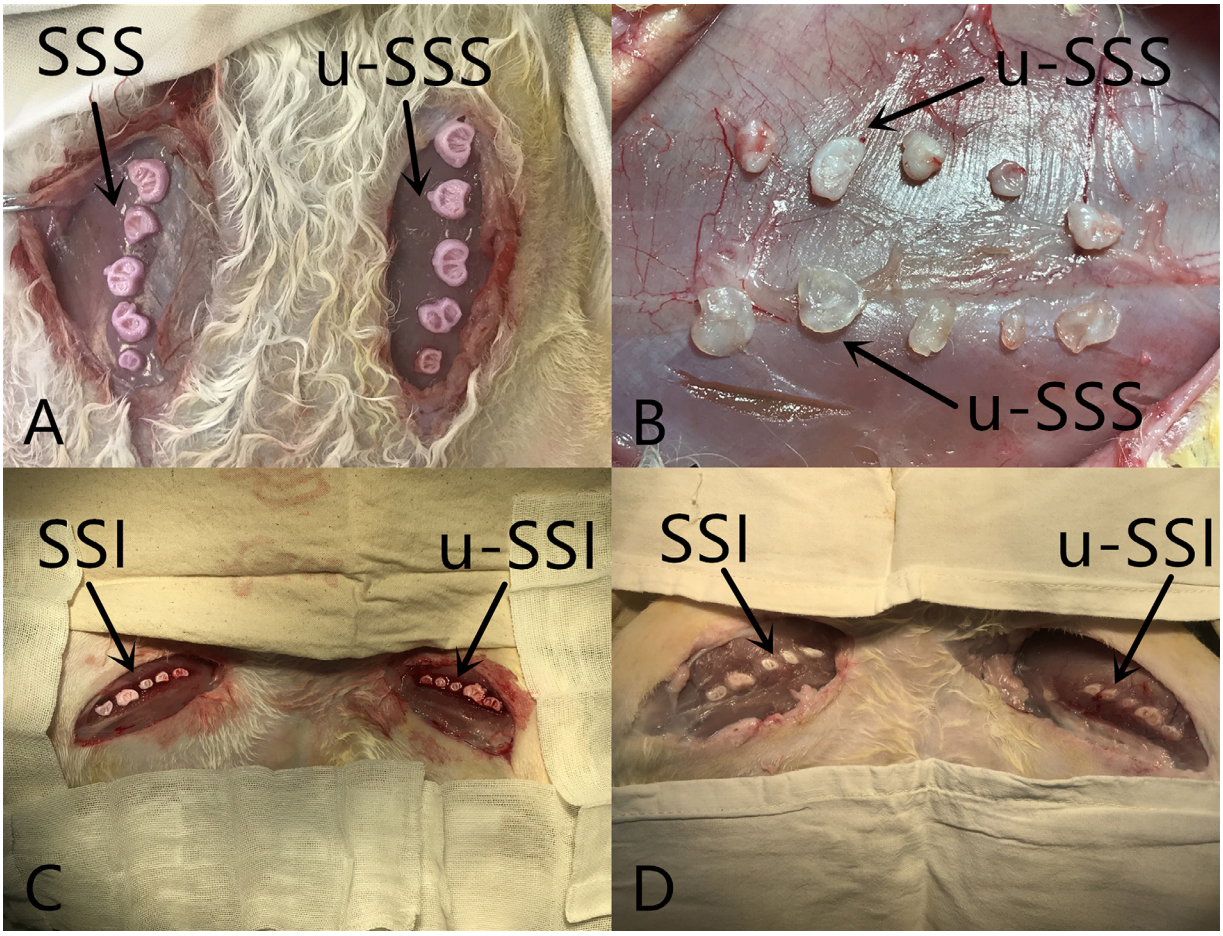

Figure 6. The gross morphology of tissue-engineered corpus cavernosum in vivo. After implantation of 6 months, the grafts in the subcutaneous site were completely squashed, and easily dissected from the surrounding tissues (A: before, and B: after); the grafts in the muscular site maintained the normal appearance, and grew closely with the surrounding tissue (C: before, and D: after).

architecture, and the collagen structure remained preserved; however, the normal micro-architecture of the ACSs in the other 3 groups disappeared. We considered that the micro-environment of intramuscular and subcutaneous sites may be different in blood supply, immune response, and mechanical pressure, which indicated that the intramuscular site might be a more suitable environment than the subcutaneous site for the construction of tissue-engineered corpus cavernosum. Furthermore, the autologous cell-seeded scaffold was more efficient compared to unseeded scaffolds, which would reduce local immune rejection and facilitate the integration of the graft.[27,28] Therefore, we thought the SSI group was the best option for further research. Due to CD31 and α-SMA being markedly expressed on endothelial cells and smooth muscle cells,[15,18] immunofluorescent staining and real-time PCR assay were performed and identified the presence of CD31 and α-SMA positive cells, and the mRNA level gradually increased after 1, 3, and 6 months, respectively. The results indicated that neovascularization gradually increased in the tissue-engineered corpus cavernosum over time. Combining with the integrity of the scaffold, it showed good histocompatibility of tissue-engineered corpus cavernosum.

However, there are some limitations to this study. First, in order to reduce the phenotypic variation of the seed cells, we only seeded ADSCs in the scaffolds 12 hours before implanting them in vivo. The results showed that the seed cells were mainly transformed into adipocytes; furthermore, it is uncertain whether the endothelial cells and smooth muscle cells detected were transformed from ADSCs. It is possible that the micro-vessels grow from the surrounding tissues into the graft. Therefore, if cytokines, such as VEGF and TGF-β, were added prior to the culture medium; or related lentiviral genes were transfected into the seed cells, it may improve the transformation rate of ADSCs to endothelial cells and smooth muscle cells.[29] Second, the scaffold harvested in this study was not a complete matrix. Previous studies have introduced the methods to construct the complete human acellular corpus cavernosum.[6,28] However, the protocol was difficult, including micro-arterial perfusion, urethral catheter perfusion and external diffusion; furthermore, it is a great challenge to seed cells homogeneously in a large-scale scaffold. Third, although we confirmed that the cell-seeded scaffold implanting into muscle was more suitable for the construction of tissue-engineered corpus cavernosum, its potential mechanism is still

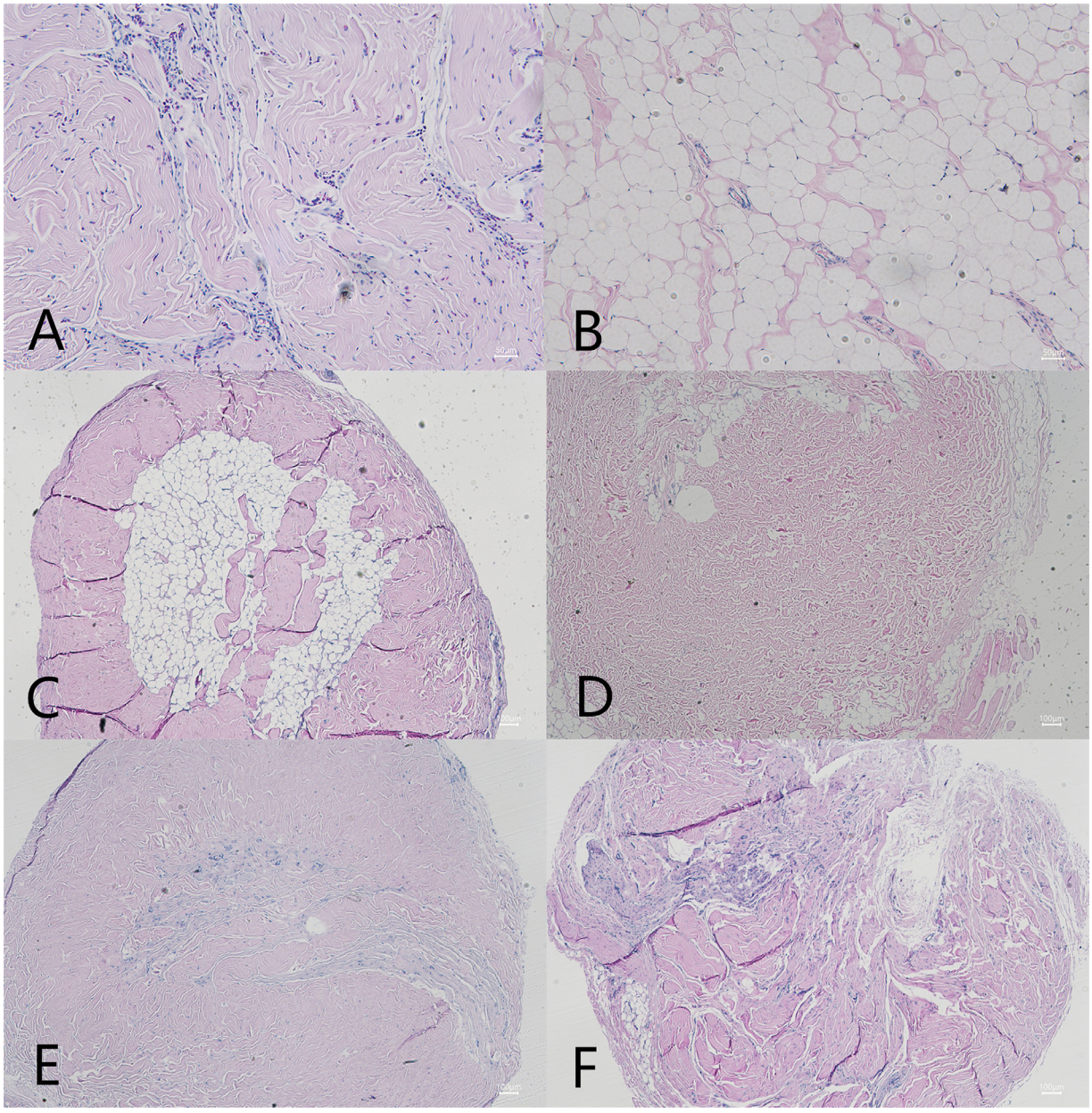

Figure 7. HE staining of tissue-engineered corpus cavernosum in vivo. After implantation of 1 months, inflammatory cell infiltration was occurred in the graft (A). After implantation of 6 months, The SSI group basically remained the normal micro-architecture, the collagen structure was still preserved, abundant adipocytes and new blood vessels can be seen (B: SSI 100x, C: SSI 40x); the normal micro-architecture of the other 3 groups (D: u-SSI, E: SSS, F: u-SSS) became disorder of cell arrangement and collagen deposition.

unknown. Further study is needed whether due to the properties of the scaffold or the recipient microenvironment.

In conclusion, we used the autologous ADSCs as seed cells, and the acellular corpus cavernosum as scaffolds, and demonstrated that the ADSCs seeded ACSs implanting back into the muscular site was a good method to construct the tissue-engineered corpus cavernosum in vivo. Despite some limitations remaining, this preliminary study could provide a better basis for the further study of tissue-engineered corpus cavernosum.

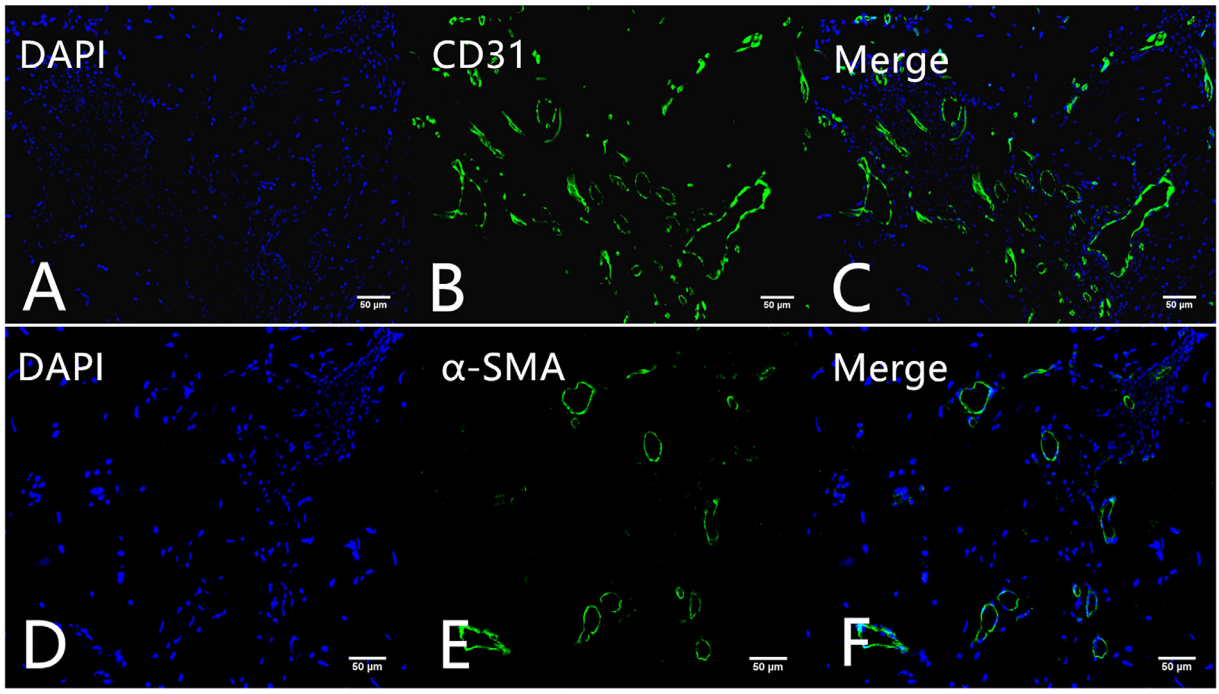

Figure 8. Immunofluorescent staining of tissue-engineered corpus cavernosum in SSI group. Immunofluorescence for CD31 (A: DAPI, B: CD31, C: merge) and α-SMA (D: DAPI, E: α-SMA, F: merge) was positive, indicating the existence of endothelial cells and smooth muscle cells, respectively.

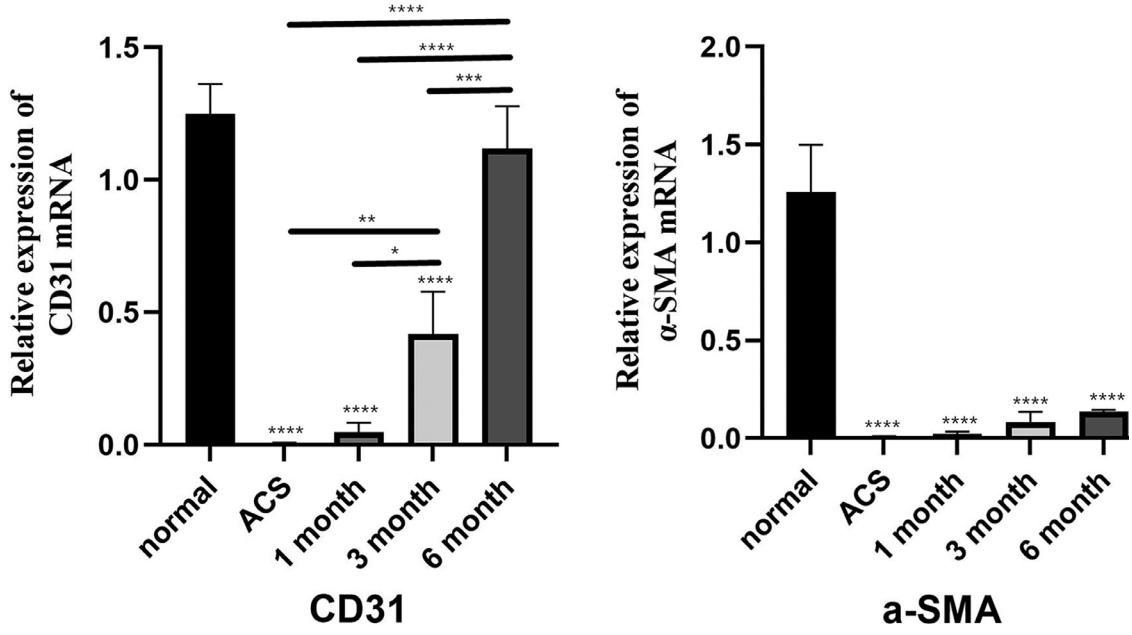

Figure 9. The real-time PCR assay was performed to detect the mRNA expression of CD31 and α-SMA in the SSI group. The results showed that CD31 and α-SMA mRNA were expressed in normal native tissue and tissue-engineered corpus cavernosum but not in ACSs. Furthermore, the quantitative expression levels of CD31 and α-SMA were gradually increased with the extension of implantation time. **** means $P < .0001$, * means $P < .05$.

Corresponding Author: Liqiang Liu, MD, Plastic Surgery Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No 33, Ba Da Chu Road, Shijingshan District, Beijing 100144, China. Tel: +86-010-88772064; Fax: +86-010-88772064; E-mail: liuliqiang@psh.pumc.edu.cn

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STATEMENT OF AUTHORSHIP

Conceptualization, Liqiang Liu and Zilong Cao; Methodology, Zilong Cao and Hu Jiao; Investigation, Zilong Cao, Hu Jiao, Cheng Gan, and Jia Tian; Writing – Original Draft, Zilong Cao.; Writing – Review & Editing, Liqiang Liu, Zilong Cao, and Bing Han; Formal Analysis, Tiran Zhang; Supervision, Liqiang Liu.

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