Effect of Adipose-derived Mesenchymal Stem Cells Combined With Urinary Bladder Matrix Scaffold on the Structure and Function of Autografted Rat Ovaries

Yanyan Xing (✉ 773791143@qq.com)
Fudan University

Wei Zhao
Changzheng Hospital: Shanghai Changzheng Hospital

Wen Li
Changzheng Hospital: Shanghai Changzheng Hospital

Research

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Abstract

**Background:** Ovarian transplantation has unique advantages in the preservation of female fertility, especially in young women with cancer who need chemotherapy. However, a large number of follicles are lost because of ischemia during ovarian tissue transplantation. While it has been reported that adipose-derived mesenchymal stem cells (ADSCs) accelerate angiogenesis, the transplanted ADSCs usually diffuse quickly from the target tissue. Urinary bladder matrix (UBM) is an extracellular matrix biomaterial that has a complete basement membrane and provides a foundation for transplanted cells to anchor, migrate, and function. In this study, ADSCs on UBM scaffolds (UBM/ADSCs) were transplanted during ovary autotransplantation in rats to test whether collagen/ADSCs have a better therapeutic effect than transplantation of ADSCs alone.

**Method:** A total of 30 rats were divided into 5 groups of 6 rats in each. untreated-control, oophorectomy, autograft, autograft + ADSCs (ADSC) and autograft + UBM/ADSCs(UBM/ADSC). 28 days after ovary autografting, follicle number, serum concentrations of follicular stimulating hormone and anti-Mullerian hormone and apoptosis rate were also estimated. At 7and 28 days post ovary autografting, angiogenesis was detected. The estrous cycle recovery was measured. The results were analyzed using one-way analysis of variance (ANOVA) and Tukey test, and the means were significantly different at P< 0.05.

**Results:** The number of both growing follicles and primordial follicles in rats in the ADSC/UBM and ADSC groups was significantly higher than that in rats in the autograft group (P <0.05). Follicle stimulating hormone levels in rats in the ADSC/UBM group were significantly decreased and anti-Müllerian hormone levels increased compared to control rats (P <0.05). Apoptosis rate in the UBM/ADSC group was lower than the autograft group (P<0.05). The angiogenesis was accelerated following ADSC/UBM transplantation. Rats in the ADSC/UBM and ADSC groups showed better estrous cycle recovery than rats in the autograft group (P<0.05).

**Conclusions:** UBM increases the retention of ADSCs in ovaries and contributes to long-term restoration of ovarian function. UBM/ADSC transplantation may be a promising candidate for ovarian transplantation.

**Background**

The incidence of cancer among young women has increased in recent years [1, 2], and ovarian function may be affected by radiotherapy and chemotherapy treatment options [3]. Ovarian tissue preservation remains an important procedure for fertility preservation in prepubertal girls [4]. Ovarian tissue cryopreservation and transplantation techniques have been successfully applied in clinical practice, with the first pregnancy after reimplantation in an orthotopic site reported in 2004. The number of live births has reached more than 130 by 2017, with a logarithmic increase over the past 2 years [5]. However, this procedure still needs to be optimized because significant follicular loss has been observed during grafting [6]. The most important challenges in ovarain transplantation are tissue ischemia and reperfusion (I/R) injury [7]. ADSCs have been transplanted to reduce the effects of I/R on ischemic...
tissues of the heart, kidneys [8], skin [9] and liver [10]. Malek et al. [11] demonstrated that ADSC transplantation improves the function of rat grafted ovaries through angiogenesis acceleration; however, ADSC therapy is restricted by insufficient settlement of transplanted cells in the target tissue [2]. The cells usually diffuse quickly to the surrounding organs or tissues, and the interplay of biological factors, including inflammation, apoptosis, and ischemia of the injected areas, make cell retention more difficult in the target tissue [12]. The application of scaffolds for the delivery and support of grafted cells represents a promising method for retaining stem cells in grafted organs [13]. Urinary bladder matrix (UBM), a decellularized extracellular matrix (ECM), is used clinically in a variety of applications. Clinical indications of commercialized UBM include the reinforcement of abdominal wall repair [14], management of diabetic ulcers [15], and management of deep wounds [16]. The immune microenvironment created by the UBM alters the presence of various cytokines and growth factors that can contribute to stem cell differentiation and tissue regeneration [14]. Therefore, in the present study, ovary autografting under the rat kidney capsule was performed and ADSCs in UBM were transplanted to the graft site, and the endocrine function and structure of the autografted ovaries were investigated.

Materials And Methods

Animals

All the animal experiments were examined and approved by the ethics committee of Shanghai Public Health Clinical Center (public health ethical examination 2019-A018-02). Eight-week old female Sprague-Dawley rats, with normal 4–5 day estrous cycles, were housed under standard conditions of 12 h light and 12 h dark cycles at 26°C, and were fed sufficient amounts of food and water in the SPF level animal room of the Second Military Medical University. Rats were randomly distributed into five experimental groups: 1. Control group (n = 6): the normal group without autograft (untreated), 2. oophorectomy group (n = 6): rats were ovariectomized, 3. Autograft group (n = 6): rats with autograft only, 4. ADSC group (n = 6): autograft with ADSC treatment, 5. ADSC/UBM group (n = 6): autograft with ADSC/UBM treatment.

ADSCisolation and culture

Rat ADSCs were isolated and cultured as previously described [34]. Briefly, adipose tissues were collected from the inguinal area of Sprague-Dawley male rats at the age of 8-10 weeks. The tissues were digested with 0.25% (v/v) collagenase type I (Gibco, USA) at 37°C for 30 min. The suspension was centrifuged at 250 × g for 20 min to separate floating adipocytes from the SVF. The SVF was suspended with high-glucose Dulbecco's modified Eagle's medium (HG-DMEM; Gibco), containing 10% fetal bovine serum (v/v) (Hyclone, USA), 50 U/ml penicillin (Gibco) and 50 mg/ml streptomycin (Gibco) in a humidified environment with 5% CO₂ (v/v) at 37°C. The culture medium was changed every 48-72 h until the cells reached 90% confluence. Isolated cells were fibroblast-like under light microscopy and attached to the bottom of the plate (Fig. 2C).

Differentiation of ADSCsandflow cytometry analysis
The ability of isolated cells to differentiate into osteocytes and adipocytes was evaluated as described previously [35]. In brief, to attain osteogenic differentiation, cells were plated in 24-well plates in an osteogenic differentiation medium containing HG-DMEM, and the medium was changed every 3 days [36]. After 2 weeks, cells were fixed and stained with Alizarin Red Suzhou Saiye Biotechnology [37].

Adipogenic differentiation of ADSCs was performed as previously described using Oil Red O staining Suzhou Saiye Biotechnology [37]. To determine cellular characteristics, cultured ADSCs were labeled with appropriate fluorescein isothiocyanate (FITC), phycoerythrin (PE), or allophycocyanin (APC)-conjugated primary antibodies for 30 min at 48°C and analyzed by flow cytometry (Becton Dickinson, USA). The antibodies detected were CD90, CD73, CD45, CD44, CD34, and CD11b (BD Pharmingen, USA).

Preparation of the urinary bladder matrix

The UBM was derived from porcine urinary bladder provided by ZhuoRuan Medical Technology Co., Ltd. (Suzhou, China). Briefly, excess adipose and collagen connective tissue was removed from the urinary bladder, and a rectangular-shaped sheet was formed by opening the bladder from the neck to the dome region. The tunica serosa, tunica muscularis externa, tunica submucosa, and muscularis mucosa were removed by mechanical delamination, leaving only the basement membrane and tunica propria intact. The remaining tissue was then soaked in deionized (DI) water and the layers of the urinary bladder that remained constituted the UBM. The UBM appeared white and consisted of soft flakes (Fig. 2A). Under a scanning electron microscope, the UBM appears loose and porous, and has a high porosity and a wide range of pore diameters, which are conducive to the colonization and proliferation of cells (Fig. 2B).

Suspended ADSCs on the UBM and observation

ADSCs (1×10^6) were suspended on the UBM approximately 3×3 mm^2, for 48 h, whereafter the ADSC/UBM fragments were studied by scanning electron microscopy (SEM). For SEM analysis, the ADSC/UBM fragments were dehydrated in a graded series of ethanol and dried with hexamethyldisilane 21 as described previously [38]. In this study, under a scanning electron microscope, flattened cells were well developed with extended cytoplasmic processes, and filopodia on UBM were observed (Fig. 2D).

Ovarian autografting

For the autograft, rats in the ADSC and ADSC/UBM groups were anesthetized with intraperitoneal injections of 3% pentobarbital sodium at a dose of 0.1 ml/100g. Under aseptic conditions, a bilateral ovariectomy was performed and the ovary was resected. The ovarian tissue was divided into two parts: a small incision was made in the ipsilateral renal capsule, and the two separated ovarian tissues were pushed under the renal capsule. Aliquot suspensions (40 ml) were injected into the core of the ovaries using 0.33 mm (29G) needles on BD Ultra-FineTM 1.0-ml disposable insulin syringes (Becton Dickinson and Company, Franklin Lakes, NJ, USA)[13]. For the autograft group, 40 ml of PBS was injected per ovary tissue. For the ADSC group, 40 ml PBS with 1 × 10^6 ADSCs was injected per ovary tissue. The ADSC/UBM group received 1 × 10^6 ADSCs in 40 ml PBS suspended on 3×3 mm^2 UBM. The muscles and skin were sutured in an interrupted fashion.
Estimation of the number of follicles

Twenty-eight days post-transplantation, rats were anesthetized and the ovaries were removed from the renal capsule, fixed in Bouin's fixative for 24 h, and dehydrated using ascending concentrations of ethanol. Ovaries were embedded in paraffin and sectioned at 5 μm, and morphological grading and follicle counts were performed following hematoxylin and eosin staining (Merck, Darmstadt, Germany). Every fifth section obtained from each ovarian paraffin block was evaluated to avoid a consecutive assessment of the same follicle; follicles were counted in ovaries from each rat in each group. Follicle assessment and follicle counting were performed according to the accepted published standards [39]. Primordial follicles were defined as oocytes surrounded by a single layer of flat granulosa cells. All other follicles at more advanced stages of maturation were grouped and defined as growing follicles. All counted primordial, primary, secondary, and tertiary follicles were classified as normal or atretic according to the following criteria: (i) primordial, single layer of flattened pre-granulosa cells; (ii) primary, single or few layers of cuboidal granulosa cells where the antrum is absent; (iii) secondary, multiple layers of cuboidal granulosa cells where a multifocal antrum is present; (iv) tertiary, multiple layers of cuboidal granulosa cells where a single antrum is present.

Immunofluorescence

At 7 and 28 days post-ovary autograft, CD31 was detected using immunohistochemical techniques. For this purpose, rats were anesthetized as mentioned above, and the ovaries were removed, fixed in Bouin's fixative for 24 h, and dehydrated using ascending concentrations of ethanol (70-100%). Slides were deparaffinized in xylene and rehydrated with decreasing concentrations of ethanol. The slides were steamed in citrate buffer (10 mM, pH 6.0) for 30 min for antigen retrieval and exposed to 3% hydrogen peroxide for 30 min. Slides were blocked in 5% BSA for 1 h and incubated with primary antibodies against CD31 (Bioworld, USA) overnight. For immunofluorescence analysis, slides were labeled with CD31 and stained with Cy3-labeled anti-rabbit IgG secondary antibodies (1:800) at 37°C for 45 min. Nuclei were counterstained with Hoechst 33342 (1:200; Sigma-Aldrich). Images were acquired sequentially on a fluorescent microscope at 200× magnification (Nikon Eclipse Ti-S).

Assessment of the apoptosis rate through terminal deoxynucleotidyl transferase-mediated deoxyuridinetriphosphate nick end-labelling (TUNEL) analysis

Twenty-eight days after ovary autografting, the DNA damage rate was estimated through TUNEL analysis of the paraffin-embedded sections based on the manufacturer's instructions (In Situ Cell Death Detection Kit; Roche). Briefly, 5-mm-thick sections were deparaffinized, rehydrated, and incubated in 3% hydrogen peroxide (H₂O₂; Merck) for 10 min. After washing with PBS, digestion with 20 mg/mL proteinase K for 30 min at 37°C was carried out. After washing twice with PBS, the sections were incubated with reactive TUNEL mixture for 60 min at 37°C in a humidified dark chamber. The sections were then washed with PBS and incubated with a liquid 3-diaminobenzidine substrate for 10 min.

Hormone assay
Twenty-eight days after transplantation, blood samples were collected and centrifuged at $3000 \times g$ for 5 min, and the levels of serum anti-Mullerian hormone (AMH) and follicle-stimulating hormone (FSH) were measured using enzyme-linked immunosorbent assay (ELISA) kits (Xi Tang, China) according to the manufacturer's instructions.

**Vaginal smear examination**

Seven days post-autograft, sterile pipettes and sterile normal saline were used to gently wash the vaginal wall of each rat. Then, the cells were smeared onto a clean glass slide and observed under a light microscope (BX51; Olympus) at 100× magnification.

**Data analysis**

Data were statistically analyzed using SPSS software (version 19) and one-way analysis of variance (ANOVA) and Tukey's test, and the means were considered significantly different at $P < 0.05$.

**Results**

**Characteristics of ADSCs**

Flow cytometry revealed that approximately 90% of the cells were negative for CD34, CD11b, and CD45, and positive for CD105, CD73, and CD90, indicating a high purity of ADSCs (Fig. 1A-J). Alizarin Red and Oil Red O staining showed that the isolated cells could differentiate into osteoblasts and adipocytes (Fig. 1K-L).

**Appearance of transplanted ovarian tissue**

On the 28th day post-operation, the kidney tissue was exposed along the original back incision to assess the transplanted ovaries. All ovarian tissues in the three autograft groups survived, and new blood vessels grew from the kidney to the graft on the surface. The autograft ovarian tissues were fresh and alive. The blood supply in rats in the ADSC and ADSC/UBM groups was more abundant than that of rats in the autograft group. There was more neovascularization in rats in the ADSC/UBM group (Fig. 3).

**The number of follicles**

The mean number of primordial and growing follicles was evaluated and counted in all groups 28 days post-autograft. The primordial follicle numbers in the ADSC and ADSC/UBM groups improved compared to those in the autograft group ($P < 0.05$), and the number of primordial follicles in ADSC/UBM rats increased significantly compared to that in ADSC rats. The outcome of growing follicles in rats in each group was similar. However, the follicle numbers in rats in each transplantation group was significantly lower than that in rats in the control group ($P < 0.05$; Fig. 4).

**Immunohistochemistry with CD31**
On day 7 post-autograft, CD31 expression in rats in the ADSC group increased significantly compared to that in rats in the autograft group. When rats were transplanted with ADSC/UBM, CD31 expression increased significantly compared to that in rats that received ADSCs only (P < 0.05; Fig. 5). On day 28 post-autograft, CD31 positive cells were further increased in rats in each group, and the expression in ADSC/UBM rats was increased significantly compared to that in rats in the other two groups (P < 0.05).

**Apoptosis rate**

The apoptotic rate was calculated using Image Pro Plus 6 software (Media Cybernetics, Silver Spring, USA). When transplanted with ADSCs, the apoptotic rate decreased compared to that in the autograft group (P > 0.05). When transplanted with ADSC/UBM, apoptosis was decreased significantly compared to the other two groups (P < 0.05; Fig. 6).

**Table 1.** Follicular stimulating hormone and anti-Mullerian hormone levels and the mean recovery rate of the estrous cycle in different groups of rats 28 days post post-ovarian tissue autograft

| Group           | FSH (ng/ml) | AMH (ng/ml) | Starting day of estrous cycle (days) |
|-----------------|-------------|-------------|-------------------------------------|
| Autograft       | 13.64±1.97<sup>bc</sup> | 0.60±0.33<sup>bc</sup> | 12±0.82<sup>b</sup> |
| ADSC            | 7.47±2.34<sup>ac</sup> | 0.87±0.28<sup>ac</sup> | 10±0.51<sup>a</sup> |
| ADSC/UBM        | 6.38±1.45<sup>abc</sup> | 0.98±0.32<sup>abc</sup> | 10±0.34<sup>a</sup> |
| Oophorectomy    | 29.14±3.84<sup>ab</sup> | 0.40±0.11<sup>ab</sup> | --- |

Values are means ±SD. <sup>a</sup>Compared with the autograft group; <sup>b</sup>Compared with the ADSC group, P < 0.05; <sup>c</sup>Compared with the Oophorectomy group, P < 0.05. (one-way ANOVA and Tukey's test, P < 0.05).

**Hormone assay and vaginal cytology**

A significant increase in the serum concentration of FSH was detected in rats in the autograft group compared with to rats in the control group, although it significantly decreased in rats in the ADSC/UBM group when compared to rats in the autograft group (P < 0.05). The serum concentration of AMH significantly decreased in rats in all the surgery groups compared to control rats (P < 0.05), and concentrations in rats in the ADSC and ADSC/UBM groups were significantly higher than those in rats in the autograft group (P < 0.05; Table 1). The estrous cycle recovered in all rats that underwent surgery, but the starting day of the estrous cycle was significantly more rapid in rats in the ADSC/UBM group than in rats in the autograft group (P < 0.05; Table 1).

**Discussion**

Angiogenesis plays an important role in tissue development, repair, and regeneration. In many diseases, such as myocardial and peripheral ischemia, diabetic ulcers, retinal diseases, and chronic wounds, the
pathophysiological problem lies in the decreased blood supply [17], which induces the death of tissue cells. The rapid formation of new capillaries is essential for tissue engineering and regenerative medicine. Capillaries deliver essential nutrients and oxygen to the cells and remove waste. Similarly, adequate blood perfusion is essential for the survival and functional life of the transplanted ovaries. How to quickly restore the blood supply of the grafts, promote the formation of new blood vessels, and reduce the loss of ovarian follicles is the key to the success of ovarian transplantation. In recent years, stem cell therapy has become a promising and advanced scientific research area [18], and has already shown potential application in several diseases, such as diabetes mellitus [19], heart failure [20], and disorders of the nervous system [21]. ADSCs, isolated within the stromal vascular fraction (SVF) [22] in a less invasive and reproducible manner, were demonstrated to differentiate into the adipogenic lineage, and their multipotency is suitable for ectodermic and endodermic tissue repair [23]. Evidence suggests that ADSCs induce neoangiogenesis during tissue repair and wound healing. They secrete factors such as vascular endothelial growth factor (VEGF), Hepatocyte growth factor (HGF), and Basic fibroblast growth factor (bFGF) [24, 25], which can stimulate the differentiation of ADSCs into endothelial cells. Recently, ADSCs were confirmed to be able to induce angiogenesis and restore the number of ovarian follicles in ovary transplantation in mice [11]. However, the seed cells quickly diffused away from the target organ, while local inflammation and ischemia further decreased the viability of the remaining stem cells, which resulted in a low survival rate of the transplanted cells [26]. Many researchers have reported that ADSCs undergo massive cell loss after injection, and a large number of engrafted cells die within 1 month after transplantation [27]. Using biomaterials, ADSCs can infiltrate the constructed scaffold and differentiate into specialized newborn young cells, and the combination of ADSCs and elastin-like recombinamers (ELR)-based hydrogels have been reported to induce the formation of new blood vessels within the biomaterials, thus providing nutrients and oxygen support [28]. UBM, a decellularized ECM, is used clinically in a variety of applications, and current evaluations of UBM composition have shown that it is composed primarily of collagens, interspersed with proteoglycan ECM, growth factors, and cytokines [29]. UBM can support multiple tissues and cell types [30] and provide a suitable niche for transplanted cells to anchor, migrate, and function [31]. In this study, we demonstrated that ADSCs grow well on the UBM, and the effect of ADSCs combined with the UBM on ovarian tissue grafted into a rat model was analyzed after ovary autotransplantation. The results of this study suggest that ADSCs improve angiogenesis in ovarian grafts 7 and 28 days post-transplantation. Additionally, the effects of ADSC/UBM were found to increase graft survival by triggering angiogenesis and reducing apoptosis. The present study confirmed that ADSCs trigger the angiogenic process, which can initiate the formation of new blood vessels either by sprouting, intussusception, or elongation via the incorporation of circulating endothelial cells. In the context of the physiological angiogenic process of folliculogenesis, most of these processes are effective. Ovarian tissue grafts are exposed to ischemic damage during the post-transplantation period until the vasculature develops. Vascular connections between the host and an ovarian strip grafted into the murine ovary were observed 5 days after transplantation [32]. To determine whether the addition of ADSCs and the UBM had a beneficial effect on vascular recruitment and limited the period of tissue hypoxia, transplanted fragments were analyzed 7 and 28 days after grafting. The results showed that graft vascularization was effectively improved as early as three days post-transplantation. Using cell-
specific CD31 immunohistochemistry, the data confirmed the proangiogenic effect of ADSC/UBM, which modulated the follicular number and altered the fibrosis and apoptosis index. In this study, follicular morphology was evaluated in several sections using light microscopy, as previously described [33]. The results showed that, in rats in the ADSC/UBM group, the density of CD31-positive cells was highest after 7 and 28 days compared to rats in the autograft and ADSC-only groups. There is probably a relationship between the presence of VEGF, FGF, and IGF in the antral follicles and corpus lutea with angiogenesis in the cortex of mature ovaries [42]. The production of these factors by the transplanted ADSCs [43] may lead to increased angiogenesis in the cortex of grafted ovaries, which is essential for folliculogenesis and the production of steroids [44,45]. UBMs can promote the attachment of exogenous stem cells, recruit stem cells from the host, and provide an ideal microenvironment for their proliferation and differentiation.

Conclusions

This study demonstrated, for the first time, that the UBM increases the retention of ADSCs in ovaries and contributes to long-term restoration of ovarian function, including estrus cycles, hormone levels, and follicle numbers. ADSC transplantation on UBMs improved the outcome of rat ovarian transplantation. UBM/ADSC transplantation is a promising candidate for ovarian transplantation.

Abbreviations

ADSCs: Adipose-derived mesenchymal stem cells; UBM: urinary bladder matrix; I/R: ischemia and reperfusion; SVF: stromal vascular fraction; FSH: follicular stimulating hormone; AMH: anti-Mullerian hormone; VEGF: vascular endothelial growth factor; HGF: hepatocyte growth factor; bFGF: basic fibroblast growth factor; ELR: elastin-like recombinamers.

Declarations

Consent for publication

Not applicable.

Availability of data and materials

Available when requested.

Competing interests

The authors declare that they have no competing interest.
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Authors' contributions

WL, FY, and JZ conceived and designed the experiments. YX and HD performed the experiments and analyzed the data. YX wrote the main manuscript. All authors reviewed the manuscript.

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Authors' information (optional)

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70:7–30.
2. Sun M, Wang S, Li Y, Yu L, Gu F, Wang C, et al. Adipose-derived stem cells improved mouse ovary function after chemotherapy-induced ovary failure. Stem Cell Res Ther. 2013;4:80.
3. Kawamura K, Cheng Y, Sun Y-P, Zhai J, Diaz-Garcia C, Simon C, et al. Ovary transplantation: to activate or not to activate. Hum Reprod. 2015;30:2457–60.
4. Ata B, Chian RC, Tan SL. Cryopreservation of oocytes and embryos for fertility preservation for female cancer patients. Best Pract Res Clin Obstet Gynaecol. 2010;24:101–12.
5. Donnez J, Dolmans MM. Fertility preservation in women. N Engl J Med. 2018;378:400–1.
6. Donnez J, Dolmans MM, Diaz C, Pellicer A. Ovarian cortex transplantation: time to move on from experimental studies to open clinical application. Fertil Steril. 2015;104:1097–8.
7. Shiroma ME, Botelho NM, Damous LL, Baracat EC, Soares-Jr JM. Melatonin influence in ovary transplantation: systematic review. J Ovarian Res. 2016;9:33.
8. Deng S, Zhou X, Ge Z, Song Y, Wang H, Liu X, et al. Exosomes from adipose-derived mesenchymal stem cells ameliorate cardiac damage after myocardial infarction by activating S1P/SK1/S1PR1 signaling and promoting macrophage M2 polarization. Int J Biochem Cell Biol. 2019;114:105564.
9. Zhou Z-Q, Chen Y, Chai M, Tao R, Lei Y-H, Jia Y-Q, et al. Adipose extracellular matrix promotes skin wound healing by inducing the differentiation of adipose derived stem cells into fibroblasts. Int J Mol Med. 2019;43:890–900.
10. Apostolou KG, Papanikolaou IG, Katselis C, Feretis T, Kletsas D, Konstadoulakis MM, et al. Undifferentiated adipose tissue stem cell transplantation promotes hepatic regeneration, ameliorates
histopathologic damage of the liver, and upregulates the expression of liver regeneration- and liver-specific genes in a rat model of partial hepatectomy. Stem Cells Int. 2018;2018:1393607.

11. Shojafar E, Mehranjani MS, Shariatzadeh SMA. Adipose-derived mesenchymal stromal cell transplantation at the graft site improves the structure and function of autografted mice ovaries: a stereological and biochemical analysis. Cytotherapy. 2018;20:1324–36.

12. Smets FN, Chen Y, Wang L-J, Soriano HE. Loss of cell anchorage triggers apoptosis (anoikis) in primary mouse hepatocytes. Mol Genet Metab. 2002;75:344–52.

13. Su J, Ding L, Cheng J, Yang J, Li X, Yan G, et al. Transplantation of adipose-derived stem cells combined with collagen scaffolds restores ovarian function in a rat model of premature ovarian insufficiency. Hum Reprod. 2016;31:1075–86.

14. Sadtler K, Sommerfeld SD, Wolf MT, Wang X, Majumdar S, Chung L, et al. Proteomic composition and immunomodulatory properties of urinary bladder matrix scaffolds in homeostasis and injury. Semin Immunol. 2017;29:14–23.

15. Frykberg RG, Cazzell SM, Arroyo-Rivera J, Tallis A, Reyzelman AM, Saba F, et al. Evaluation of tissue engineering products for the management of neuropathic diabetic foot ulcers: an interim analysis. J Wound Care. 2016;25:18–25.

16. Parcells Al, Abernathie B, Datiashvili R. The use of urinary bladder matrix in the treatment of complicated open wounds. Wounds. 2014;26:189–96.

17. Shang Q, Bai Y, Wang G, Song Q, Guo C, Zhang L, et al. Delivery of adipose-derived stem cells attenuates adipose tissue inflammation and insulin resistance in obese mice through remodeling macrophage phenotypes. Stem Cells Dev. 2015;24:2052–64.

18. Safford KM, Hicok KC, Safford SD, Halvorsen Y-DC, Wilkison WO, Gimble JM, et al. Neurogenic differentiation of murine and human adipose-derived stromal cells. Biochem Biophys Res Commun. 2002;294:371–9.

19. Song B-Q, Chi Y, Li X, Du W-J, Han Z-B, Tian J-J, et al. Inhibition of notch signaling promotes the adipogenic differentiation of mesenchymal stem cells through autophagy activation and PTEN-PI3K/AKT/mTOR pathway. Cell Physiol Biochem. 2015;36:1991–2002.

20. Lin T-M, Tsai J-L, Lin S-D, Lai C-S, Chang C-C. Accelerated growth and prolonged lifespan of adipose tissue-derived human mesenchymal stem cells in a medium using reduced calcium and antioxidants. Stem Cells Dev. 2005;14:92–102.

21. Reichenberger MA, Mueller W, Hartmann J, Diehm Y, Lass U, Loellensperger E, et al. ADSCs in a fibrin matrix enhance nerve regeneration after epineural suturing in a rat model. Microsurgery. 2016;36:491–500.

22. Atesci AA, Avci CB, Tuglu MI, Ay NPO, Eronat AC. Effect of different dentin conditioning agents on growth factor release, mesenchymal stem cell attachment and morphology. J Endod. 2020;46:200–8.

23. Goldman KN, Chenette D, Arju R, Duncan FE, Keefe DL, Grifo JA, et al. mTORC1/2 inhibition preserves ovarian function and fertility during genotoxic chemotherapy. Proc Natl Acad Sci U S A.
24. Patel ZS, Mikos AG. Angiogenesis with biomaterial-based drug- and cell-delivery systems. J Biomater Sci Polym Ed. 2004;15:701–26.

25. Zakrzewski W, Dobrzynski M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. Stem Cell Res Ther. 2019;10:68.

26. Trivedi HL, Vanikar AV, Thakker U, Firoze A, Dave SD, Patel CN, et al., Human adipose tissue-derived mesenchymal stem cells combined with hematopoietic stem cell transplantation synthesize insulin. Transplant Proc. 2008;40:1135-9.

27. Yan W, Guo Y, Tao L, Lau WB, Gan L, Yan Z, et al. C1q/Tumor necrosis factor-related protein-9 regulates the fate of implanted mesenchymal stem cells and mobilizes their protective effects against ischemic heart injury via multiple novel signaling pathways. Circulation. 2017;136:2162–77.

28. Fernandes M, Valente SG, Sabongi RG, dos Santos JBG, Leite VM, Ulrich H, et al. Bone marrow-derived mesenchymal stem cells versus adipose-derived mesenchymal stem cells for peripheral nerve regeneration. Neural Regen Res. 2018;13:100–4.

29. Warren R, Chestnut MH, Wong TK, Otte TE, Lammers KM, Meili ML. An improved method for the isolation and cultivation of human scalp dermal papilla cells: maintenance of extracellular matrix. Ann N Y Acad Sci. 1991;642:436–8.

30. Dhar S, Yoon ES, Kachgal S, Evans GRD. Long-term maintenance of neuronally differentiated human adipose tissue-derived stem cells. Tissue Eng. 2007;13:2625–32.

31. Liang J, Zhang H, Kong W, Deng W, Wang D, Feng X, et al. Safety analysis in patients with autoimmune disease receiving allogeneic mesenchymal stem cells infusion: a long-term retrospective study. Stem Cell Res Ther. 2018;9:312.

32. Mazini L, Rochette L, Admou B, Amal S, Malka G. Hopes and limits of adipose-derived stem cells (ADSCs) and mesenchymal stem cells (MSCs) in wound healing. Int J Mol Sci. 2020;21:1306.

33. Bellei B, Migliano E, Tedesco M, Caputo S, Papaccio F, Lopez G, et al. Adipose tissue-derived extracellular fraction characterization: biological and clinical considerations in regenerative medicine. Stem Cell Res Ther. 2018;9:207.

34. Mazo M, Arana M, Pelacho B, Prosper F. Mesenchymal stem cells and cardiovascular disease: a bench to bedside roadmap. Stem Cells Int. 2012;2012:175979.

35. Seo YS, Ko IO, Park H, Jeong YJ, Park J-A, Kim KS, et al. Radiation-induced changes in tumor vessels and microenvironment contribute to therapeutic resistance in glioblastoma. Front Oncol. 2019;9:1259.

36. Argentati C, Morena F, Bazzucchi M, Armentano I, Emiliani C, Martino S. Adipose stem cell translational applications: from bench-to-bedside. Int J Mol Sci. 2018;19:3475.

37. Freytes DO, Martin J, Velankar SS, Lee AS, Badylak SF. Preparation and rheological characterization of a gel form of the porcine urinary bladder matrix. Biomaterials. 2008;29:1630–7.
38. Zhang L, Zhang F, Weng Z, Brown BN, Yan H, Ma XM, et al. Effect of an inductive hydrogel composed of urinary bladder matrix upon functional recovery following traumatic brain injury. Tissue Eng Part A. 2013;19:1909–18.

39. Boccafoschi F, Habermehl J, Vesentini S, Mantovani D. Biological performances of collagen-based scaffolds for vascular tissue engineering. Biomaterials. 2005;26:7410–7.

40. Demeestere I, Simon P, Emiliani S, Delbaere A, Englert Y. Orthotopic and heterotopic ovarian tissue transplantation. Hum Reprod Update. 2009;15:649–65.

41. Tilly Jl. Ovarian follicle counts—not as simple as 1, 2, 3. Reprod Biol Endocrinol. 2003;1:11.

**Figures**
Figure 1

Characterization analyses of ADSCs. (A-J) Flow cytometric characterization of ADSCs. ADSCs were negative for CD45, CD11b, and CD34 and positive for CD105, CD90 and CD73. (K-L) Adipogenic and osteogenic differentiation of ADSCs, (K) Oil Red O and (L) Alizarin Red staining.
Figure 2

UBM and ADSC observation. (A-B) Appearance and structure of the UBM; (C) Early passage ADSCs; (D) ADSC/ UBM observation by SEM.
Figure 3

28 days after ovarian autografting. (A-C) Transplanted ovarian tissue under the renal capsule; (a-c) Ovarian tissue removed from the renal capsule.
Figure 4

Follicle evaluation. (A–E) Histological analysis of rat ovaries by H&E staining; (F) Mean number of primordial and growing follicles. *Compared with the control group, P < 0.05; # compared with the autograft group, P < 0.05.
Figure 5

CD31 expression. (A) Immunohistochemical staining of CD31 in rats 7 days post-autograft; (B) CD31 expression in rats 7 and 28 days post-autograft. * P < 0.05.
Figure 6

Apoptosis rate analysis. (A) TUNEL assays were performed 28 days post-autograft. (B) Apoptosis rate 28 days post-autograft. * P < 0.05.