Biocompatibility of polypropylene mesh scaffold with adipose-derived stem cells

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Abstract. In this study, we investigated the rejection of the synthetic patch and human tissues in the host. We observed the growth of adipose-derived stem cells (ADSCs) cultured with polypropylene mesh *in vitro*. The results of flow cytometry showed that the expression of CD44, CD73, CD90, CD45, CD14 and CD34 was 98.54, 95.32, 98.49, 1.21, 3.01 and 2.14%, respectively. ADSCs were isolated from rabbit subcutaneous adipose tissue after collagenase digestion, filtration and centrifugation. The ADSCs of passage 3 were seeded onto the polypropylene mesh scaffolds. New Zealand White female breeder rabbits were implanted with polypropylene mesh, ADSC-fixed polypropylene mesh in the abdomen. After 4 weeks, adhesion was performed and the erosion of the mesh was evaluated. It was found that polypropylene mesh, ADSC-fixed polypropylene mesh all had different degrees of corrosion, and adhesion, but polypropylene mesh was more corroded. ADSC-fixed polypropylene mesh induced a milder chronic inflammation response compared with polypropylene, had significantly lower scores for inflammation (t=11.083), and had significantly higher scores for neovascularization (t=14.362) and fibroblastic proliferation (t=15.979). The relative amount of VEGF mRNA was significantly lower for ADSC-fixed polypropylene compared with the other polypropylene meshes (t=94.6). In conclusion, polypropylene mesh scaffold with ADSCs exhibit excellent cellular compatibility and are promising in clinical practice.

Introduction

In recent years, the incidence of female pelvic floor dysfunction (PFD) has been on the increase, and seriously affects the physical and mental health as well as the life quality of women. Taking advantage of the synthetic graft materials to conduct pelvic reconstruction surgery is a common method used to treat PFD (1). However, avoiding the tissue rejection and erosion of the prosthetic mesh and human tissue remains to be resolved.

The adipose-derived stem cells (ADSCs) are at present intensively investigated, have the ability of self-renewal and multiple differentiation potential, are easily obtained, and are suitable as the seed cells of tissue engineering (2). As the candidate seed cells of tissue engineering, ADSCs were selected for the present study to investigate the biocompatibility of ADSCs and polypropylene mesh to provide a fundamental basis for future research and application.

Materials and methods

Experimental materials. Experimental materials included, Collagenase I (Sigma-Aldrich, St. Louis, MO, USA); CD90, CD34, CD44, CD45 and CD106 monoclonal antibodies (Long Island Biotec., Shanghai, China); and RT-PCR kit (Takara, Tokyo, Japan). PCR primers used were produced by Sangon Biotech Co., Ltd. (Shanghai, China). Polypropylene mesh was purchased from Huantai Goldenlake Carapace Products Co., Ltd. (Zibo, China).

Main instruments. The instruments used were: Constant temperature CO_2 cell incubator (Thermo Fisher Scientific, Inc., Waltham, MA, USA); super-clean worktable (Shanghai Medical Instruments Co., Ltd., Shanghai, China); micrography system, TE300-type inverted phase contrast microscope (Nikon, Tokyo, Japan); flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA); and Image-Pro Plus image processing software version 7.0 (Media Cybernetics, Rockville, MD, USA).

Laboratory animals. Thirty healthy female New Zealand rabbits of 3-4 months of age, and 1.5-2.2 kg of weight were raised by Shanghai Shengwang Experimental Animals Breeding Co. Ltd. (Shanghai, China) with animal production license no. SCXK (H) 2007-0007 and use license no. SYXK (H) (2006-0010). The animals were raised at 25˚C and relative humidity of 50%. During the experiment, the animals were treated as per the regulations specified in the Guidance on Humane Treatment of Laboratory Animals.

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Methods

Isolation and culture of ADSCs. Amobarbital sodium at 3% was used for intravenous anesthesia of rabbits, and the skin preparation and disinfection were conducted in inguinal region. Subsequently, 25 g of subcutaneous fat was taken by the physicians and preserved in DMEM containing 5% FBS. The tissues were cut into sections by scissors after being washed by streptomycin and phosphate-buffered saline (PBS) solution. The cut-out adipose tissues were then added with 0.1% of collagenase solution I of 20 ml, placed in the incubator at 37˚C for 60 min and centrifuged at 1,500 x g for 10 min under the condition that the centrifugal radius was 13.5 cm, and finally the cells were resuspended by adding the culture solution to the supernatant which was carefully taken from the collagenase solution. The cells were then inoculated into the culture dish at the cellular concentration of $1x10^6$/ml. The culture dish with the cells was inoculated and cultured in the incubator at 37˚C with a volume fraction of 5% of CO_2. The cells (80%) were progressed to passage after cell fusion. The 3rd-5th generation of cells were chosen to conduct the experiments.

Stem cell surface markers detected by flow cytometry. The 3rd generation of cells in the logarithm growth period were taken and centrifuged at 750 x g for 5 min at the centrifugal radius of 13.5 cm, after which the supernatant was discarded. Cells were centrifuged for another 5 min after being washed with PBS. The pre-cooled PBS was used to adjust the cell density until it reached $1x10^6$/ml. Cell suspension was then prepared and flow cytometry was used to detect ADSC-related antigens including CD90, CD34, CD44, CD45, CD14 and CD73. The characteristics of stem cells were confirmed by detecting the expression level of CD molecules.

Implantation test. Thirty New Zealand female rabbits were divided into 2 groups randomly and then implanted with polypropylene mesh and ADSC composite polypropylene mesh, respectively. After the routine anesthesia, the hypogastric region of rabbits was disinfected and the longitudinal incision was placed in the median line of hairless region. The skin and subcutaneous tissues were cut apart to reveal the rectus abdominis muscle, followed by covering with polypropylene mesh of 1x1 cm and ADSC composite polypropylene mesh on the surface of rectus abdominis muscle. Finally the thread residue was retained in the position where the patch was implanted as a mark and the incision was sutured by silk threads. The rabbits in sham-operated group were not implanted with any material, and the remaining operating procedures were identical with those in the above-mentioned group. After 4 weeks, a general observation was conducted on the mesh adhesion and the corrosion occurred in the sacrificed rabbits. Ten mesh-muscular tissue samples were taken out of the same position of each rabbit, and a part of the samples were observed under a light microscope (Olympus, Tokyo, Japan) after being stained by hematoxylin and eosin and made into pathological sections. All sections were given a histological score by referring to the standard formula by Valentin *et al* (3). As for the remaining samples, the expression levels of vascular endothelial growth factor (VEGF) mRNA was detected by RT-PCT.

Detection of VEGF mRNA expression level with RT-PCR. Total RNA of tissues was extracted in accordance with the total RNA extraction kit, and electrophoresis was used to detect the quality of RNA. RT-PCR was performed in accordance with the kit introduction for 30 cycles, and PCR amplification products were extracted with 5 μl in each product and identified as per 1.5% of agarose gel electrophoresis. The Bio-Rad Transilluminator was used for imaging and Quantity One software version 4.6 was used for analysis (both from Bio-Rad Laboratories, Inc., Hercules, CA, USA). The ratio of light absorption value of internal control band to the light absorption value of target band was used to calculate the expression quantity of genes.

Statistical analysis. SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA) was used to conduct statistical analysis, and the quantitative data were expressed as mean ± standard deviation (SD). The comparison on histological score in each group was tested using a t-test, the comparison on the expression level of VEGF mRNA was tested by Wilcoxon. The comparison on proliferation rate was tested by χ^2. $P \leq 0.05$ was set as the statistically significant difference.

Results

In vitro culture form and identification of ADSCs. On the first day when ADSCs were inoculated into the culture dish, only a small number of spindle cells adhered to the wall and they were of different sizes. Two days later, the quantity of cells adhering to the wall were markedly increased and spindle or triangle in shape. Three days later, the clone cells began to form and grow in a specific direction. After the first generation of cells, the cells distributed in a swirling pattern were observed. After the passage, the morphologic change of cells had little change and they were still distributed in the shape of long spindle, and their proliferation capacity was exuberant (Fig. 1).

According to the detection results as shown by flow cytometry, the strongly positive expression of specific mesenchymal stem cell markers included CD90, CD44 and CD73 of 98.54, 95.32 and 98.49%, respectively. In contrast, the strongly positive expression of hematopoietic cell-related surface markers included CD45, CD34 and CD14 of 1.21, 3.01 and 2.14%, respectively (Fig. 2).

Gross observation. There were 4 cases (26.7%) in the polypropylene mesh group and 2 cases (13.3%) in the ADSC composite polypropylene mesh group that suffered from erosion reaction due to the penetration of materials into the rectus abdominis muscle 4 weeks after the polypropylene mesh or composite polypropylene mesh was implanted into the rabbits. There were 2 cases in polypropylene mesh group that suffered from mesh and abdominal adhesions (Fig. 3).

Histopathological observation of implanting position. A large number of inflammatory cells, a small number of fibroblast and new vessels began to form around the polypropylene mesh 4 weeks after the implantation. The small number of inflammatory cells, large number of fibroblast and new vessels began to form around the ADSC composite polypropylene mesh (Fig. 4). The inflammatory reaction around the opening

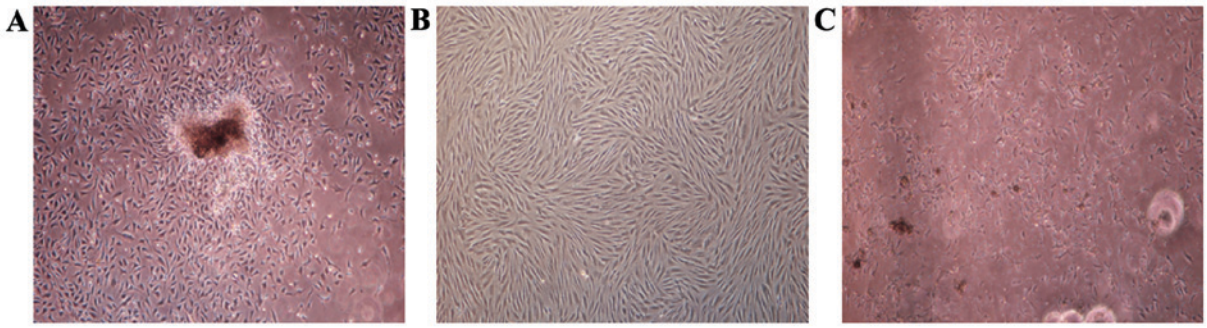

Figure 1. The growth of adipose stem cells under an inverted microscope. (A) Primary culture (magnification, x20). (B) Culture for 7 days (magnification, x20). (C) Second generation of cells (magnification, x20).

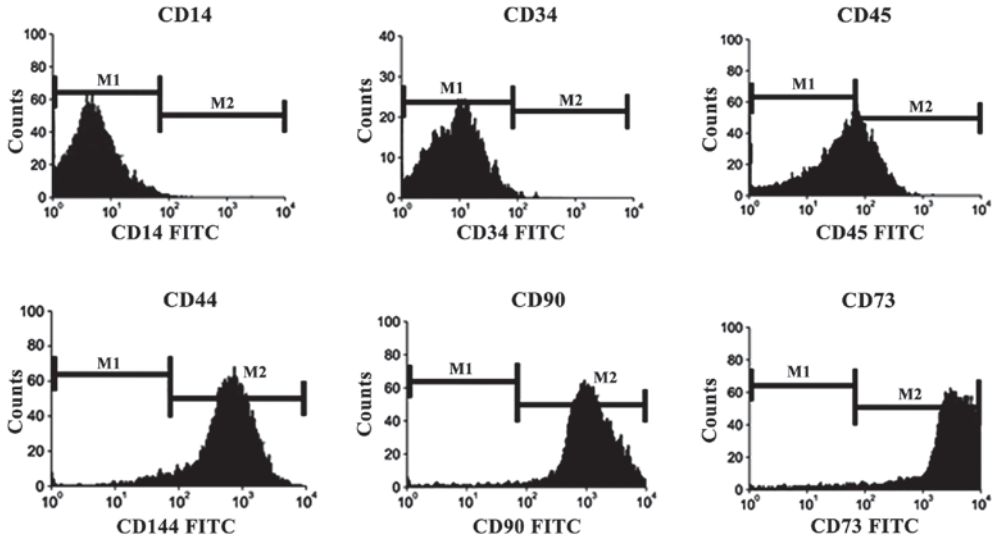

Figure 2. ADSC surface markers with flow cytometry. M1 G1 phase representing cells; M2 G2 phase representing cells. ADSCs, adipose-derived stem cells.

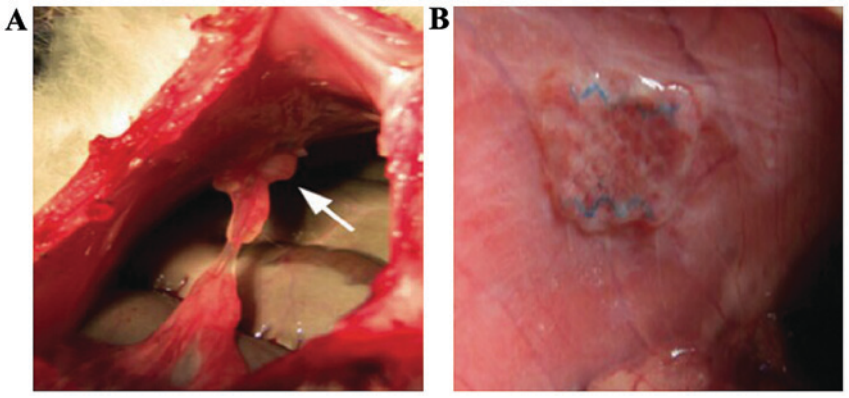

Figure 3. The gross observation of the net tablets after 3 weeks of implantation in the rectus rectus muscle. (A) Polypropylene mesh and abdominal adhesions. (B) ADSC composite polypropylene mesh penetrated into the rectus abdominis muscle. ADSCs, adipose-derived stem cells.

of polypropylene mesh became moderate-severe 4 weeks after the operation, and the inflammation score around the opening of polypropylene mesh was the highest, and the difference had statistical significance when it was compared with ADSCs composite polypropylene mesh (Table I) ($P<0.05$). The new vessels around the ADSC composite polypropylene mesh began to form and proliferation rate of fibroblasts was higher, and the difference had statistical significance when it was compared with polypropylene mesh ($P<0.05$), and there was no fibroblasts proliferation in the sham-operated group (Fig. 2).

Expression level of VEGF mRNA. Expression of VEGF mRNA was detected in the tissues around the implant materials. The expression level of VEGF mRNA of ADSC composite polypropylene mesh was significantly higher than that of VEGF mRNA of polypropylene mesh ($t=94.6$, $P<0.05$) (Fig. 5).

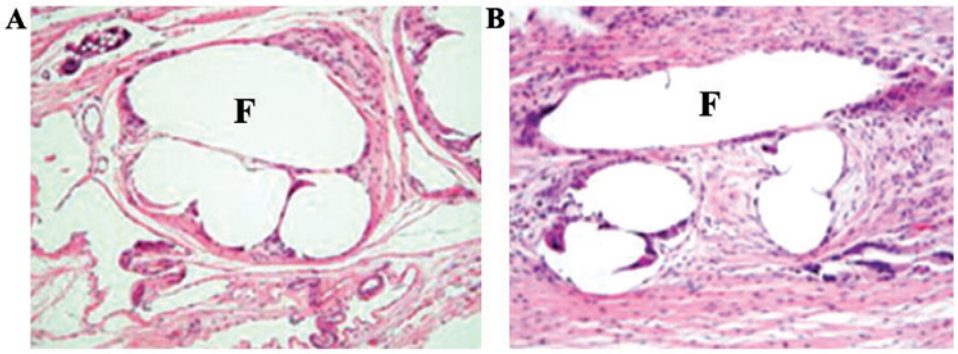

Figure 4. Histopathological changes in each group after implantation for 4 weeks (H&E; magnification, x200). (A) ADSC composite polypropylene mesh. (B) Polypropylene mesh. (F) Mesh fibers. H&E, hematoxylin and eosin; ADSCs, adipose-derived stem cells.

Table I. Histological score standard (mean ± SD).

Index	ADSCs composite polypropylene mesh score	Polypropylene mesh score	T-value	P-value
Cellular infiltration	2.3±0.4	2.9±0.5	9.372	0.003
Cell type	1.5±0.0	1.8±0.1	7.114	0.018
Inflammatory reaction	0.6±0.1	1.1±0.2	11.083	0.001
Angiognesis	2.6±0.3	1.7±0.0	14.362	<0.001
Fibrosis	2.2±0.2	0.9±0.1	15.979	0.001

ADSCs, adipose-derived stem cells.

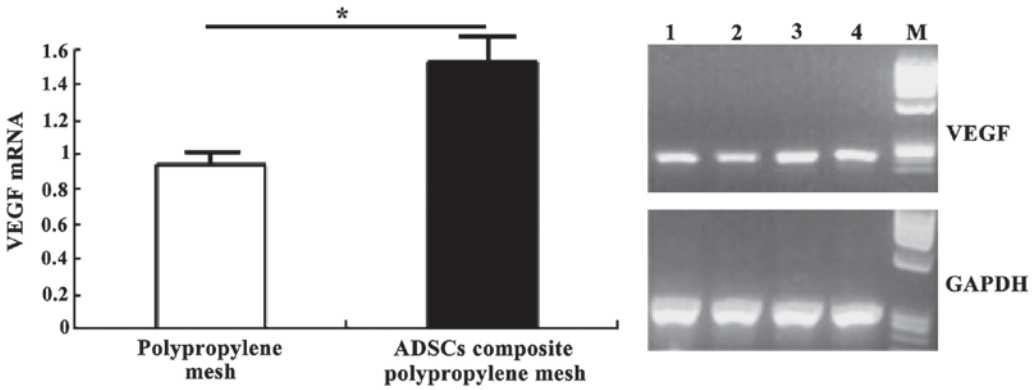

Figure 5. The expression level of VEGF mRNA. M, marker. Lanes 1 and 2, polypropylene mesh; lanes 3 and 4, ADSC composite polypropylene mesh. *P<0.05. VEGF, vascular endothelial growth factor; ADSCs, adipose-derived stem cells.

Discussion

Female PFD is a disease resulting from the defects or degradation, injury and dysfunction of pelvic support structure, which seriously influences the life quality of female patients. In recent years, the synthetic patch has been adopted for pelvic reconstruction surgery. The satisfactory patch should be sterile, non-absorbing and have no allergy and inflammatory reaction, and the Prolift polypropylene mesh produced by Johnson & Johnson (New Brunswick, NJ, USA) is mainly adopted to conduct pelvic reconstruction, but polypropylene materials can inevitably result in rejection, erosion and many other problems (3-5). Therefore, reduction of the complications from implant materials has become a challenge, which needs to be solved for pelvic reconstruction surgery.

The stem cell treatment international research and can be divided into embryonic stem cells and adult stem cells according to the survival stage. In 2001, ADSCs were isolated from adipose tissues by the way of suction lipectomy (6), and further research indicated that the stem cells can be divided into bone, cartilage, and muscle cells. The ADSCs were characteristic of: i) The adipose tissues can be easily obtained and causes little damage to the patients; ii) ADSCs have no immunological rejection and involve no medical ethics problems; and iii) the stem cells expand rapidly, thus a large number

of cells can be obtained in a short time (7-9). At present, the most common methods to acquire seed cells include enzyme digestion assay and tissue block culture method, thus the tissue block culture method was adopted by the authors in this experiment to extract the ADSCs (10-12). We could see that the clone cells began to grow in a specific direction after three days of inoculated culture. The flow cytometer was adopted to detect the strongly positive expression of specific mesenchymal stem cell markers including CD90, CD44 and CD73. However, the expression of hematopoietic cell-related surface markers which included CD45, CD34 and CD14 was 1.21, 3.01 and 2.14%, respectively, which excludes the possibility that the hematopoietic cells are from stem cells and provides a fundamental basis for the next experiment.

The most common methods to investigate the histocompatibility of biological materials include direct *in vivo* implantation method and composite cell culture *in vitro*. However, there are few fundamental research reports on ADSC composite polypropylene mesh inside the body. We studied the histological response when ADSC composite mesh was implanted inside the body of the hosts to provide a theoretical basis for the application of ADSC composite polypropylene mesh in pelvic reconstruction.

The inflammatory response was immediately triggered after the mesh materials were implanted into the body, and the severe inflammatory response would not only lead to the swelling and necrosis of part of the tissues but also to severe fibrosis, thus resulting in the deformation and retraction of the mesh, hardness increased in part of the tissues, and reduction of compliance and mesh erosion (13,14). We found that the mesh fabrics were surrounded by more inflammatory response cells around the polypropylene mesh in the fourth week after the implantation. The inflammatory response of tissues in ADSC composite polypropylene mesh was mild with more macrophages and lower fibrosis degree. In addition, the adhesion between the mesh and surrounding tissues were lighter, which proved that it has better histocompatibility.

The constructs of tissue engineering is a process where the stem cells are divided into the mature terminal tissues after the stem cells and the complexes of scaffold materials are implanted inside the body. The cell-carrier complex itself has no nutritional source, and the tissues of small size may acquire nutrition through the penetration of surrounding tissue fluid so that the tissues can develop blood circulation with surrounding tissues. However, upon the construction of tissue-engineered tissues of large size, the ability for the cells to develop blood circulation just through penetration effect is very limited, and the cells compounded on scaffold materials died before the development of blood circulation (15). There are many factors promoting the regeneration of new vessels, and VEGF is one of the factors which have been studied thoroughly and is the most complex factor. The results of this experiment showed that there are more VEGF in the surrounding tissues of ADSC polypropylene mesh and the degree of vascularization is higher. It may be related to the generation of VEGF and various kinds of vascular growth factors which are induced by ADSCs so that more new vessels are produced, which suggested that ADSC polypropylene mesh has better biocompatibility.

In conclusion, ADSCs composite polypropylene mesh has better biocompatibility and can reduce earlier inflammatory reaction and fibrosis degree, reduce the tissue adhesion and reduce the occurrence rate of mesh-related complications.

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