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Experimental study on application of polypropylene hernia of fat stem cells in rats

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Abstract. – OBJECTIVE: An inguinal hernia is the most common type of external abdominal hernia. Normal surgery utilized polypropylene mesh to fix a hernia, but often leads to adverse effects including pain and foreign body sensation. Adipose-derived stem cells are pluripotent stem cells that can be differentiated into adipocytes, chondrocytes, skeletal myoblast and osteoblast. The compatibility of stem cells with absorbent material mesh or cellular biology mesh has been studied. This study investigated the application of adipose-derived stem cells coated polypropylene mesh in treating an inguinal hernia.

MATERIALS AND METHODS: Adipose-derived stem cells were separated and cultured from adipose tissues of SD rats. Flow cytometry was utilized to determine antigen subtype including CD90, CD34, CD44, and CD45 on the cell surface. Those cells were then induced to be differentiated into adipocytes, osteocytes and chondrocytes. Adipose-derived stem cells coated polypropylene mesh was then prepared and implanted under a muscular layer of rat peritoneal cavity. The occurrence of hematoma and routine blood indexes were examined.

RESULTS: Adipose-derived stem cells showed attached growth with satisfactory proliferation ability. Flow cytometry results showed positive rates for CD90, CD44, CD34, and CD45 at 98.2% ± 1.4%, 97.6% ± 2.3%, 54.3% ± 6.3%, and 13.4% ± 4.2%, respectively. Adipose-derived stem cells had the potency of differentiation. The application of novel mesh decreased macrophage number and lymphocytes ($p < 0.01$) but not leukocytes or neutrophils ($p > 0.05$).

CONCLUSIONS: Adipose-derived stem cells coated polypropylene mesh can decrease the inflammation post inguinal hernia and can be used as one novel biomaterial in treating an inguinal hernia.

Key Words:

Adipose-derived stem cells, Polypropylene mesh, Inguinal hernia.

Introduction

An inguinal hernia is one common abdominal external hernia and is normally used pure suture repair, but with a high recurrence rate[1]. Currently, an artificial synthetic material is commonly used as the mesh to repair a hernia without tension to decrease postoperative recurrence rate[2]. However, adverse effects including local pain, foreign body sensation and decreased peritoneal wall compliance may occur after the implantation of artificial synthetic materials especially polypropylene mesh[3]. Those adverse effects are mainly caused by the (sub-) acute inflammation after using artificial synthetic mesh and local hyperplasia of fiber tissues, leading to compression of nerves/vessels, and further foreign body sensation and chronic pains[4].

Adipose-derived stem cells (ADSCs) are one type of stem cells with self-renewal and pluripotency for differentiation[5] and can be stably passed toward multiple generations[6], therefore owing multiple features for tissue engineering materials. Previous studies[5,7,8] have shown the potency of ADSCs to differentiate into multiple cell types including adipocytes, chondrocytes, myoblasts and osteoblasts. Moreover, the endothelial growth factor secreted by ADSCs could facilitate angiogenesis[9]. Meanwhile, ADSCs had sufficient supply, can be re-sampled from animals with rapid proliferation, and are easy for collection and separation. Previous study[10] has shown that implanted ADSCs could differentiate toward fibroblasts, vascular endothelial cells and squamous epithelial cells. Moreover, ADSCs could secrete various collagen, fibronectin and growth factors via paracrine manner, to accelerate tissue healing[11]. The compatibility between various stem cells and absorbance material scaffold or cellular biology scaffold, as stem cells showed proliferation on various mate-

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rials including polylactic acid (PLA), polylactic acid/polyethylene glycol copolymer (PLA-PEG), polylactic acid/polyglycolic acid copolymer (PLGA), collagen, bone tissue scaffold and cell-free pericardium[12]. This work thus investigated the possibility of using ADSCs-coated polypropylene mesh to replace pure polypropylene mesh in treating inguinal hernia.

Materials and Methods

Reagents and Equipment

CO_2 cell culture incubation was purchased from SANYO (SANYO, Tokyo, Japan); inverted microscope (Leica, Germany). Flow cytometry was purchased from Beckman (Beckman Coulter, Brea, CA, USA). FITC-labeled mouse antibody was purchased from Abcam Biotechnology (Cambridge, MA, USA). Fetal bovine serum was purchased from HyClone (South-Logan, UT, USA) Dulbecco's Modified Eagle Medium F12 (DMEM-F12) culture medium was purchased from Gibco BRL. Co. Ltd. (Grand Island, NY, USA).

Animals

A total of 40 healthy specific pathogen free (SPF)-grade Sprague-Dawley (SD) rats (20 males and 20 females, body weight 220-250 g, aging 4-6 weeks) were purchased from Experimental Animal Unit, Zhejiang University. Animals were acclimated for 2 weeks before experiment and were then randomly divided into polypropylene mesh group and ADSCs-coated polypropylene mesh group (n=20).

ADSCs Separation and Collection

0.4% sodium amobarbital was intraperitoneally for anesthesia. Subcutaneous adipose tissues were separated from inguinal site under sterilized conditions. Adipose tissues were rinsed in phosphate-buffered saline (PBS) and cut into small pieces, and were cultured in DMEM-12 medium containing 10% fetal bovine serum. The culture flask was inverted for 20 min and adipose tissues continuously incubated with medium changed every 3 days.

Identification of ADSCs

Under an inverted phase-contrast microscope, both cell morphology and proliferation of ADSCs were observed. Adipocytes at 2nd generation of log-phase were adjusted to 1×10^9/l to prepare cell suspensions. Monoclonal antibody was added for 4°C incubation for 30 min, followed by PBS rinsing twice, and 1000 r/min centrifugation for 5 min. Flow cytometry was then employed to detect surface antigens including CD90, CD34, CD44 and CD45 on cell surface. Each sample was measured by three times.

Identification of Pluripotent Differentiation Ability

Induction of adipocytes: Adipocyte induction complete medium A (2 ml/well) was added for initial culture, followed by adipocyte induction complete medium B for 24 h after 3 days. The rotation of culture medium persisted for 3 cycles. When a large number of small lipid droplets occurred, adipocyte induction buffer B was used to incubate for 5 days. When the lipid droplet became larger, oil red O dye was added for observation. Osteocyte induction: Cells were seeded into 6-well plate (2 ml/well) and were incubated in CO_2 chamber. After reaching confluence of 80-90%, culture medium was discarded, followed by the addition of 2 ml osteocytes induction complete medium, which was changed every 3 days. After 4 weeks, alizarin red was stained for microscopic observation. Chondrocyte induction: After digestion, cells were induced in centrifuged tube with 1.0×10^9/l concentration. Using chondrocyte induction medium, which was changed every two days, the culture persisted for 4 weeks. After fixation, cells were cut into paraffin-based sections, which were stained by toluidine blue. Polypropylene mesh was cut into 5 mm × 5 mm pieces, each of which was seeded with 0.1 ml cell suspensions, and were seeded into 6-well plate. After 4 h, DMEM-F12 medium (8 ml) was added for incubation at 37°C with 5% CO_2 in an incubator. The medium was changed every 3 days for 2 weeks. A 1cm-length incision was made on the lateral abdominal wall of rats. Pre-coated and clean mesh was implanted under abdominal muscular layer. No extra suture or fixation was made on any mesh. The incision was sutured by absorbent silks.

Postoperative Evaluation

Twenty-eight days after surgery, the hematoma formation at surgical site was evaluated, along with routine blood parameters. 2 month later all rats were sacrificed and extracted for tissues with mesh implantation for hematoxylin-eosin (HE) staining. Number of macrophage was counted under 400× microscope.

Statistical Analysis

SPSS17.0 software package (SPSS, Inc., Chicago, IL, USA) was used to analyze all collected data, of which measurement data were presented as mean±standard deviation (SD). The Student's *t*-test was used to compare the differences between two groups. The Tukey's post-hoc test was used to validate the ANOVA for comparing measurement data between groups. $p < 0.05$ was treated as statistical significance.

Results

ACS Biology Morphology and Flow Cytometry

Under inverted phase-contrast microscope, cell growth and confluence were observed. After two generations of *in vitro* culture, most cells showed attached growth with spindle shape. Lipid droplets can be observed in certain cells (Figure 1). Further flow cytometry assay detected antigen CD90, CD34, CD44 and CD45 on the surface of ADSCs. Results (Figure 2) showed that CD90 and CD44 had positive rates at 98.2% ± 1.4%, 97.6% ± 2.3%, respectively, indicating strong positive. The positive rates of CD34 and CD45, however, were 54.3% ± 6.3%, 13.4% ± 4.2%, respectively, suggesting stem cell features.

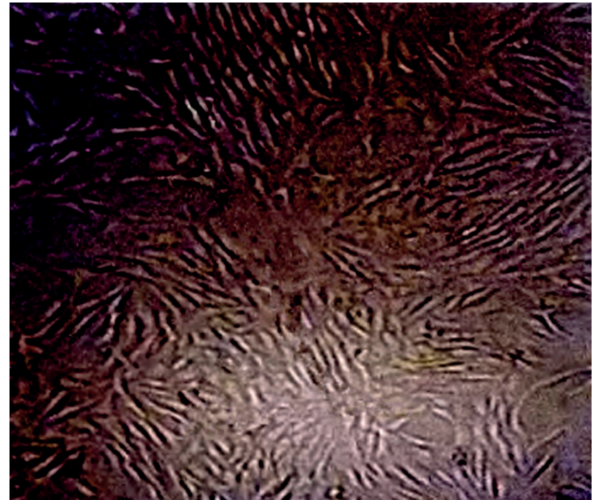

Figure 1. ADSCs morphology (×200).

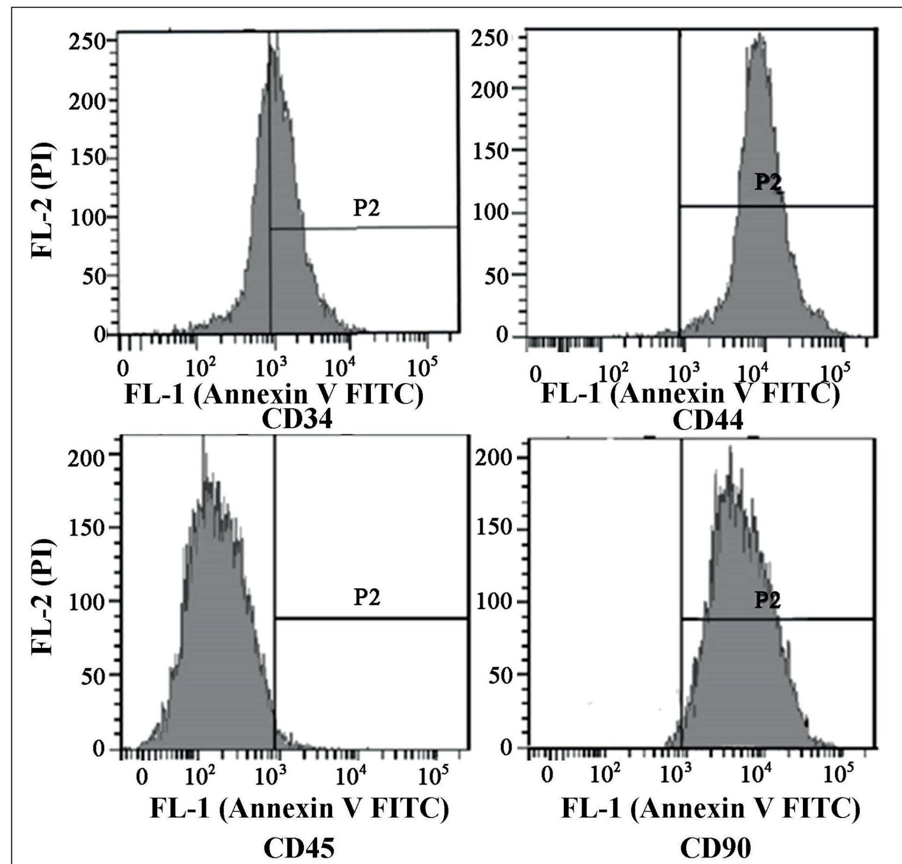

Figure 2. Flow cytometry assay for levels of surface antigen CD90, CD34, CD44 and CD45 on ADSCs.

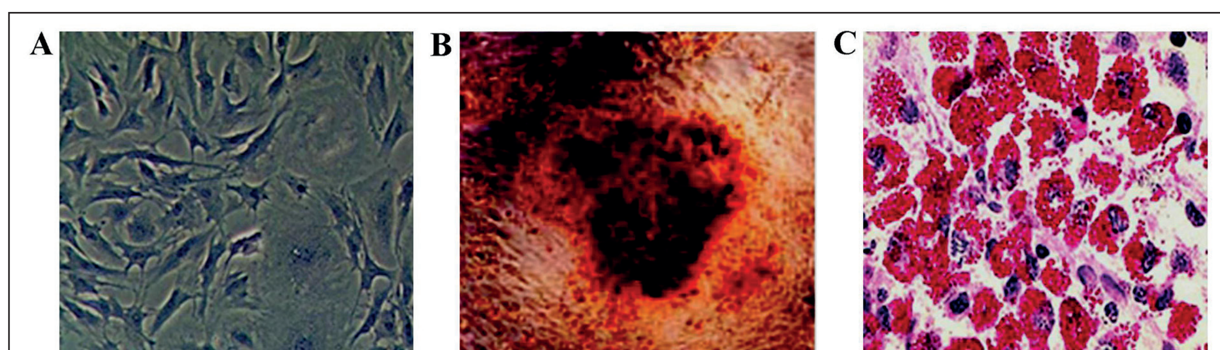

Figure 3. Identification of pluripotent differentiation potency of ADSCs. **A,** toluidine blue staining after chondrocyte induction. **B,** alizarin red staining after osteocyte induction; **C,** oil red O staining after adipocyte induction.

Detection of Pluripotent Differentiation Ability

After induction of ADSCs and staining identification, results (Figure 3) showed brown-black granules by toluidine blue staining after chondrocytes induction. The induction by osteocytes led to mineral nodules inside cells, while adipocyte induction leads to granular lipid droplet in cytoplasm, suggesting pluripotent differentiation potency of ADSCs.

Postoperative Efficacy Evaluation

Using ultrasonic examination for local edema formation of all rats after surgery, we found no significant difference of hematoma occurrence between groups ($p > 0.05$). Routine blood test showed that between pure polypropylene mesh and ADSCs coated polypropylene mesh group, no significant difference of leukocytes or neutrophils can be observed ($p > 0.05$). The number of lymphocytes, however, was significantly changed between groups ($p < 0.01$). Meanwhile, HE staining on macrophage counting revealed significantly more cells in pure polypropylene mesh group ($p < 0.01$, Table I).

Discussion

The identification of ADSCs mainly utilizes morphology and cell surface tag. Previous knowledge revealed spindle shape of most ADSCs, which are regularly arranged. Our observation fitted features of ADSCs by cell morphology. No typical and specific surface marker has been developed for ADSCs, as only CD13, CD29, CD44, and CD54 have been used to label stem cells[13]. This study selected CD90, CD34, CD44 and CD45 as cell surface markers. Results showed strong positive expression of CD90 and CD44, and lower expression of CD34 and CD45, suggesting satisfactory purity of ADSCs extraction. We also performed targeted differentiation induction on ADSCs to determine their pluripotent differentiation potency. Results showed the ability of differentiating into chondrocytes, osteocytes and adipocytes of those ADSCs, as consistent with previous studies bout inducing ADSCs[14-16]. Previous study has shown the potency of clinical application of mesenchymal stem cells and embryonic stem cells[17], whose rejection reaction, however, was continuously reported. The dis-

Table I. Hematoma and blood examination of rats using different meshes.

Index	Polypropylene mesh	ADSCs coated polypropylene mesh	*p*-value
Hematoma (Yes/No)	4/20	2/20	0.38
Red blood cell	8.72 ± 0.43	8.37 ± 0.74	0.07
Leukocyte	9.53 ± 2.10	8.23 ± 1.60	0.03
Neutrophil	1.45 ± 0.23	1.37 ± 0.39	0.43
Lymphocyte	8.21 ± 1.20	6.77 ± 1.10	$p < 0.01$
Macrophage[a]	15.8 ± 4.3	8.3 ± 3.2	$p < 0.01$

Note: [a]Macrophage number in 5 fields (×400).

covery of ADSCs brings about new insights for xenograft, as they may work as better candidates for cell therapy and tissue engineering[18,19]. This study utilized both normal polypropylene mesh and ADSCs coated materials in surgical practice. By comparison, we found by simultaneous implantation of both types of mesh under abdominal muscular layer of rats, no significant difference of hematoma incidence can be observed ($p > 0.05$). Routine blood test showed no statistically significant difference of red blood cell, leukocyte and neutrophil counts between two groups. The number of lymphocytes, however, was different between groups ($p < 0.01$). Those rats received normal polypropylene mesh implantation also had more macrophage ($p < 0.01$). Previous studies have shown that ADSCs could fuse with poly (lactic-co-glycolic acid) (PLGA) microsphere to produce adipose tissues inside nude mouse[20,21]. Such complex can grow in non-cross-linked hyaluronic acid gel to form microsphere with great treatment potency[22]. So far no other reports using ADSCs in tissue engineering have been found.

Conclusions

This study used rats as the animal model due to its relatively larger size and easy access to clinical indexes. Due to the smaller sample size and lack of surgical instruments in rodent model, few indexes have been observed to evaluate the treatment efficacy. Thus large-sample observation or clinical trial is required to substantiate our model along with long-term effects. Meanwhile, due to the limitation of animal study, the subjective uncomfortable cannot be analyzed. The clinical value of ADSCs coated polypropylene mesh thus requires further investigations.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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