Biomaterials 35 (2014) 8801–8809

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Repair of Achilles tendon defect with autologous ASCs engineered tendon in a rabbit model

Dan Deng [a, 1, 2], Wenbo Wang [a, 1], Bin Wang [a, 1], Peihua Zhang [b, *], Guangdong Zhou [a], Wen Jie Zhang [a], Yilin Cao [a], Wei Liu [a, *]

[a] Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Tissue Engineering Research, National Tissue Engineering Center of China, Shanghai, PR China
[b] College of Textiles, Donghua University, Shanghai, PR China

ARTICLE INFO

Article history:
Received 24 June 2014
Accepted 29 June 2014
Available online 25 July 2014

Keywords:
ASCs
Rabbit Achilles tendon
In vivo repair
PGA/PLA scaffold

ABSTRACT

Adipose derived stem cells (ASCs) are an important cell source for tissue regeneration and have been demonstrated the potential of tenogenic differentiation *in vitro*. This study explored the feasibility of using ASCs for engineered tendon repair *in vivo* in a rabbit Achilles tendon model. Total 30 rabbits were involved in this study. A composite tendon scaffold composed of an inner part of polyglycolic acid (PGA) unwoven fibers and an outer part of a net knitted with PGA/PLA (polylactic acid) fibers was used to provide mechanical strength. Autologous ASCs were harvested from nuchal subcutaneous adipose tissues and *in vitro* expanded. The expanded ASCs were harvested and resuspended in culture medium and evenly seeded onto the scaffold in the experimental group, whereas cell-free scaffolds served as the control group. The constructs of both groups were cultured inside a bioreactor under dynamic stretch for 5 weeks. In each of 30 rabbits, a 2 cm defect was created on right side of Achilles tendon followed by the transplantation of a 3 cm cell-seeded scaffold in the experimental group of 15 rabbits, or by the transplantation of a 3 cm cell-free scaffold in the control group of 15 rabbits. Animals were sacrificed at 12, 21 and 45 weeks post-surgery for gross view, histology, and mechanical analysis. The results showed that short term *in vitro* culture enabled ASCs to produce matrix on the PGA fibers and the constructs showed tensile strength around 50 MPa in both groups ($p > 0.05$). With the increase of implantation time, cell-seeded constructs gradually form neo-tendon and became more mature at 45 weeks with histological structure similar to that of native tendon and with the presence of bipolar pattern and D-periodic structure of formed collagen fibrils. Additionally, both collagen fibril diameters and tensile strength increased continuously with significant difference among different time points ($p < 0.05$). In contrast, cell-free constructs failed to form good quality tendon tissue with fibril structure observable only at 45 weeks. There were significant differences in both collagen fibril diameter and tensile strength between two groups at all examined time points ($p < 0.05$). The results of this study support that ASCs are likely to be a potential cell source for *in vivo* tendon engineering and regeneration.

1. Introduction

Tendon engineering offers a promising approach for repairing tendon defect without the need of harvesting autologous tendon. This concept has been approved in various *in vivo* studies using different animal models including mouse [1,2], hen [3], and pig [4]. Rabbit is the most common animal model for engineered tendon repair including patellar tendon [5] and Achilles tendon [6].

Cell source remains a major challenge in engineered tendon repair. Despite tenocytes have been successfully employed for tendon engineering and repair, they are difficult to serve as a cell source for tendon engineering due to the necessity of harvesting autologous tendon. Although dermal fibroblasts have also been employed as a cell source for engineered tendon repair *in vivo* [4], adult stem cells may have an advantage over fully differentiated cells as they possess the capability of self-renewal and multiple

* Corresponding authors. Shanghai 9th People's Hospital, 639 Zhi Zao Ju Rd, Shanghai 200011, PR China. Tel.: +86 21 2327 1699 5601; fax: +86 21 5307 8128.
E-mail addresses: phzh@dhu.edu.cn (P. Zhang), liuwei_2000@yahoo.com (W. Liu).
[1] Authors who contributed equally.
[2] Current address: Department of Dermatology, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine.

http://dx.doi.org/10.1016/j.biomaterials.2014.06.058
0142-9612/

differentiation potential, and adult stem cell engineered tendons may have better self-repair function upon injury. Furthermore, adult stem cells such as mesenchymal stem cells have the functions of immune modulation and are less immunogenic [7,8] and thus are likely to serve as an allogenic cell source.

Previously, bone marrow derived mesenchymal stem cells (BMSCs) were used for tendon engineering [9] and repair in a few animal models including the rabbit model [10–12]. Comparing to BMSCs, adipose-derived stem cells (ASCs) are more advantageous over BMSCs, because they are more convenient for harvesting, easier to be expanded with less osteogenic tendency and lower immunogenicity [13–15]. Accordingly, ASCs have been used as the cell source for the engineering and repair of bone [16], cartilage [17], skin [18] nerve [19] and myocardium [20]. ASCs have not yet been tested for their ability to engineer and repair tendon *in vivo*, although they have been shown to improve tendon healing in a horse model [21]. This study tested the effect of ASCs on engineered tendon repair *in vivo* using rabbit Achilles tendon defect as an animal model and polyglycolic acid (PGA) and polylactic acid (PLA) fibers as the scaffolds.

2. Materials and methods

2.1. Experimental design

Two groups were involved in this study, the constructs with ASCs seeded on PGA/PLA scaffolds were set as an experimental group, while scaffolds only (cell free) were prepared as a control group, all samples were *in vitro* cultured for 5 weeks followed by *in vivo* implantation to repair Achilles tendon defect in a rabbit model.

2.2. Animals

Total 30 four-week-old New Zealand White rabbits were used in this study and 10 rabbits were eventually excluded from the study due to unexpected death and severe infection at the operating sites. Animals, either male or female, in weight of 1.7–2.5 kg, were purchased from Shanghai Chuansha Breeding Factory. For this study, 15 rabbits were used for the experimental group and 4 dropped. Similarly, 15 rabbits were initially assigned to the control group with 6 rabbits dropped from the study. Animals were sacrificed at the time points of weeks 12 ($n = 3$), 21 ($n = 4$) and 45 ($n = 4$) post-surgery for the experimental group and at weeks 12 ($n = 3$), 21 ($n = 3$) and 45 ($n = 3$) for the control group. An institutional review committee of Shanghai Jiao Tong University School of Medicine approved all animal study protocols.

2.3. Fat harvesting procedure

Nuchal subcutaneous adipose tissue was harvested from the rabbits in sterile condition and placed into a 50 ml centrifuge tube containing Dulbecco's Modified Eagle Medium (DMEM, Hyclone, Logan City, UT) supplemented with 10% fetal bovine serum (FBS, Hyclone), L-glutamine (292 mg/ml), penicillin (100 U/ml), streptomycin (100 mg/ml), and ascorbic acid (50 mg/ml). The tissue samples were processed for cell isolation within 2 h post-harvest. Rabbits were returned to cages after wound closure via suturing.

2.4. Cell isolation and culture

After being thoroughly rinsed with phosphate-buffered saline (PBS) and soaked in 2.5% chloramphenicol solution for 5 min, the adipose tissues were washed again in PBS and then minced aseptically followed by enzyme digestion with 0.075% collagenase II (Worthington, Freehold, NJ) in DMEM culture medium as above described at 37 °C on a rotator as previously described [3]. The resulting cell suspension was collected after 2 h of digestion and filtered through a sterile nylon mesh (Tetko, Elmsford, N.J.) to remove tissue residues. The filtrate was further centrifuged at 1500 rpm for 5 min and cell pellets were washed with PBS and then resuspended in DMEM culture medium. The cell suspension was transferred to a 100 mm culture dish ($1 \times 10^4/cm^2$) for expansion at 37 °C in a humidified 5% CO_2 atmosphere. When cultured cells reached 80% confluence, they were detached with 0.25% trypsin-EDTA (Gibco, Grand Island, N.Y.) and subcultured at the same density ($1 \times 10^4/cm^2$).

2.5. Preparation of scaffold material

In this study, a composite scaffold was used. This scaffold was composed of an inner part of fiber scaffold and an outer part of a net. Briefly, the inner part is PGA unwoven fibers (Shanghai Ju Rui Biomaterials Company Inc, Shanghai, China) which were longitudinally arranged to form a cord in weight of 80 mg. The outside part was a net scaffold knitted with PGA and PLA fibers (Shanghai Ju Rui Biomaterials Company Inc) in a ratio of 4:2 to provide essential mechanical strength. A 5-0 absorbable surgical suture was used to seal off two ends of the composite scaffold resulting in a cord with a length of 3 cm and a diameter of 1 cm (Fig. 1). Before use, each construct was soaked in 75% ethanol for 30–60 min followed by 3 washes in PBS, and then pre-incubated in DMEM medium supplemented with 10% FBS at 37°C overnight in an incubator. Afterwards, the medium was removed and the construct was subsequently air dried for 30 min followed by ultraviolet light exposure overnight for sterization.

2.6. In vitro culture of cell-scaffold construct

The cultured ASCs at five passage were collected and re-suspended in culture medium at a density of 5×10^7 cell/ml. In a culture dish, rabbit ASCs were seeded evenly onto the composite scaffold to form cell-scaffold constructs and then kept in an incubator for 4 h, allowing complete adhesion of the cells to the scaffold material. Afterwards, culture medium was added, and the cell-scaffold construct was incubated for another 24 h. Then the cell-seeded constructs were mounted to a bioreactor to culture *in vitro* for 5 weeks with mechanical loading.

During this time period, both cell-seeded and cell-free scaffolds were subjected to a dynamic stretch loading with a frequency of 3 times per minute (10 s stretching and 10 s interval), a stretch amplitude of 1/10 length of the constructs. The loading was performed for 1 h every 12 h as previously reported [22]. The culture medium was changed every three days. Mechanical analysis and scanning electron microscopic (SEM) examination were performed on *in vitro* cultured constructs prior to *in vivo* transplantation.

2.7. Surgical procedure

After intramuscular injection of ketamine (10 mg/kg) and Lumianning (5 mg/kg) for anesthetization, an aseptic incision was made at the dorsal aspect of the right posterior leg above the ankle joint. A 2-cm-long tendon fragment was resected to create a partial tendon defect of Achilles tendon. Both ends of the severed tendon were allowed to retreat by themselves, resulting in a defect about 3 cm in length. To prevent unhealed tendon from overstretching, the cultured cell-seeded constructs with a length of 3 cm were implanted into the defect to bridge the tendon defect with 5-0 suture in the experimental group. The implantation of cell-free constructs was performed in the control group in the same way (Supplementary Fig. s1). The rabbits were kept in the cages for their free activities after surgical procedure. The animals were respectively sacrificed at weeks 12, 21 and 45 post-surgery to harvest tissue specimens for histological, transmitting electronic microscope (TEM) and biomechanical analysis.

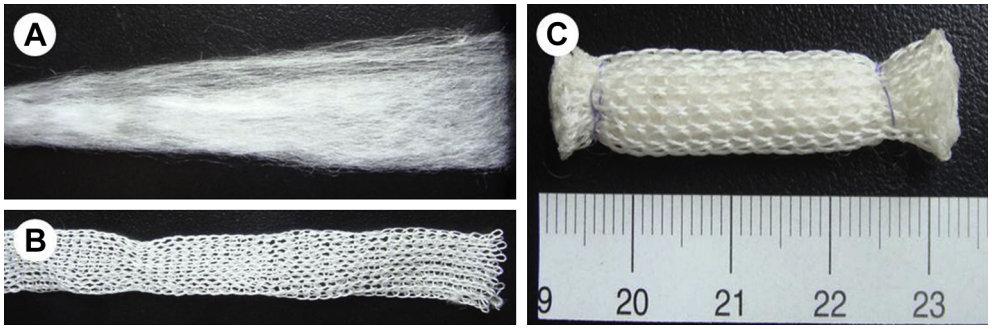

Fig. 1. Preparation of a composite tendon scaffold. The scaffold was composed of an inner part of PGA unwoven fibers (A) and an outer part of a net knitted with PGA/PLA fibers in a ratio of 4:2 (B). The outcome of assembled two parts (C).