Biomaterials 51 (2015) 43–50

Contents lists available at ScienceDirect

Biomaterials

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

Effect of mechanical stimulation on bone marrow stromal cell–seeded tendon slice constructs: A potential engineered tendon patch for rotator cuff repair

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ARTICLE INFO

Article history:
Received 9 October 2014
Accepted 25 January 2015
Available online 17 February 2015

Keywords:
Bone marrow stromal cells
Stretch
Tendon slice
Tissue engineering

ABSTRACT

Cell-based tissue engineered tendons have potential to improve clinical outcomes following rotator cuff repair, especially in large or massive rotator cuff tears, which pose a great clinical challenge. The aim of this study was to develop a method of constructing a functional engineered tendon patch for rotator cuff repair with cyclic mechanical stimulation. Decellularized tendon slices (DTSs) were seeded with BMSCs and subjected to cyclic stretching for 1, 3, or 7 days. The mechanical properties, morphologic characteristics and tendon-related gene expression of the constructs were investigated. Viable BMSCs were observed on the DTS after 7 days. BMSCs penetrated into the DTSs and formed dense cell sheets after 7 days of mechanical stretching. Gene expression of type I collagen, decorin, and tenomodulin significantly increased in cyclically stretched BMSC-DTS constructs compared with the unstrained control group ($P < 0.05$). The ultimate tensile strength and stiffness of the cyclically stretched tendon constructs were similar to the unstrained control group ($P > 0.05$). In conclusion, mechanical stimulation of BMSC-DTS constructs upregulated expression of tendon-related proteins, promoted cell tenogenic differentiation, facilitated cell infiltration and formation of cell sheets, and retained mechanical properties. The patch could be used as a graft to enhance the surgical repair of rotator cuff tears.

1. Introduction

Rotator cuff tears are one of the most common causes of shoulder pain [1]. Approximately 50% of the US population has had a degenerative rotator cuff tear by age 60 [2]. Although small rotator cuff tears have better treatment outcomes, surgical repairs of large tears show failure rates as high as 90% because of muscle contraction, decreased range of joint motion, neurovascular damage, or altered shoulder mechanics [3]. High retear rate and poor functional outcomes are common after surgical repair, and currently available scaffolds have limited ability to enhance new tendon formation [4]. Consequently, optimal treatment of rotator cuff tears should focus on preservation or restoration of rotator cuff function. However, designing functional rotator cuff tendon grafts remains a great challenge.

Augmentation of rotator cuff repair is frequently performed with acellular matrices or cell–seeded scaffolds [5–7]. Cell-based strategies in particular seem to be associated with superior results [8]. Previous work has focused on identifying an ideal cell-scaffold framework that could replace the lost tendon. Different scaffold materials such as chitosan constructs [5], injectable hydrogels [6], or knitted silk-collagen scaffolds [7] have mixed results and limited applicability, mostly because the graft strength is substantially lower compared with native rotator cuff tendons. To enhance the strength of engineered tendons, entire tendons can be decellularized and used as scaffolds.

Abbreviations: BMSC, bone marrow stromal cell; DTS, decellularized tendon slices; ECM, extracellular matrix; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IACUC, Institutional Animal Care and Use Committee; PBS, phosphate-buffered saline; RT-PCR, reverse-transcription polymerase chain reaction; UTS, ultimate tensile strength.

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http://dx.doi.org/10.1016/j.biomaterials.2015.01.070
0142-9612/

Decellularized tendon slices (DTSs) promise advantages compared with tendon allografts or synthetic materials. First, the immunogenicity and antigenicity of the tissue is reduced by extracting antigenic cells and preserving only the minimally antigenic extracellular matrix (ECM). Cell disruption and extraction of cell detritus minimizes adverse immunogenic reactions. Second, by preserving other macromolecules besides the collagenous structure, an ideal environment is provided for exogenous (seeded) cells for incorporation, metabolism, and matrix synthesis [17]. By seeding DTSs with bone marrow stromal cells (BMSCs), the process of tendon incorporation and remodeling may be hastened. Further, by preserving the natural collagenous structure, the original biomechanical strength is preserved. This method is suitable for making tendons completely cell free without changing their biomechanical properties [31].

Bioreactors in tissue engineering [9] create a controlled and sterile environment for development of engineered tissues, including tendon [10]. Bioreactors designed to provide in vitro mechanical stimulation are thought to replicate physiologic conditions and promote cell differentiation and tenogenesis [11]. Such bioreactor stimulation has been shown to improve biomechanical properties in decellularized tendon scaffolds reseeded with rabbit tenocytes [12]. However, distribution of the reseeded cells was limited to the surface, with far fewer cells in the core of the scaffold [13]. Whitlock et al. [14] increased the porosity and pore size of the tendon scaffold by using a combined solution of TritonX-100 and peracetic acid. Although cells could migrate into deeper layers of tendon tissue, the native structure and ECM microenvironment of the tendon were changed. Omae et al. [15] investigated a novel scaffold with multilayer tendon slices, but tendon slices with a thickness of 50 μm are not expected to have enough initial mechanical strength to withstand early motion and rehabilitation. Recently Qin et al. [16] demonstrated that native tendon slices with a thickness of at least 300 μm had similar biomechanical characteristics to the intact tendon bundle. DTSs with a thickness of 300 μm maintained elemental mechanical strength, inherent ultrastructure, specific proteoglycans, and multiple growth factors of tendon ECM [17]. More recent work has shown that grafts of DTSs incorporate into the host tendon, which improves biomechanical performance of the regenerated rotator cuff tendon [18]. However, in that study, the DTSs graft did not host any seeded cells; thus, the performance of cell–seeded DTSs has not yet been characterized.

The aim of this in vitro study was to develop a novel, cell–seeded patch for rotator cuff repair and to test the effect of a mechanical-loading protocol on the cell–seeded patch. The patch was a DTS construct that was seeded with viable BMSCs and then subjected to dynamic stretch culturing. We reviewed morphologic changes, expression of tendon-related genes in BMSCs, and biomechanical properties of the patches after stretch culture. We hypothesized that BMSCs could grow well on the DTS scaffold and that dynamic stretching could stimulate tenogenic differentiation of BMSCs.

2. Materials and methods

2.1. Preparation of DTSs

DTSs were prepared as previously described [17]. Briefly, Achilles tendons (length, 40 mm) were harvested from dogs euthanized for other studies approved by the Mayo Clinic Institutional Animal Care and Use Committee (IACUC). Tendons were soaked in liquid nitrogen for 10 min and then thawed at room temperature. The freeze-thaw procedure was repeated 5 times to disrupt all cells in the tendon. Each segment was embedded with optimal cutting temperature compound (Sakura Finetek USA, Inc), fixed on a cryostat (CM1850, Leica), and then longitudinally cut into 300-μm slices in succession. After washing with phosphate-buffered saline (PBS) for 30 min, DTSs were incubated in a nuclease solution (RNase [100 μg/mL] and DNase [150 IU/mL], Roche Diagnostic) for 12 h at 37 °C and then washed with PBS. Slices were frozen at −80 °C for 60 min and then lyophilized in a freeze dryer (Millrock Technology Inc). Finally, the slices were packed in a polyethylene bag and sterilized with ethylene oxide gas.

2.2. Cultivation of BMSCs

Bone marrow was harvested from 10 mixed-breed dogs (weighing 25–30 kg) used in other studies approved by the Mayo Clinic IACUC. Immediately after euthanasia, 4.0 mL of bone marrow was aspirated from the tibia using a 20-mL syringe containing 1.0 mL of heparin (50 IU/mL). The syringe contents were combined with 5.0 mL PBS and centrifuged at 1500 rpm for 5 min at room temperature. After the PBS and heparin were removed, bone marrow cells were cultured onto 4 dishes (100 mm in diameter) in 8 mL of standard medium, which consisted of minimal essential medium with Earle salts (Gibco), 10% fetal calf serum, and 1% antibiotic-antimycotic (Gibco). Bone marrow cells were incubated at 37 °C with 5% CO_2 and 100% humidity. After 5 days, the culture medium and floating cells were removed. The remaining adherent cells were defined as BMSCs [19]. The culture medium was changed every other day. BMSCs in the second passage were treated with 0.05% ethylenediaminetetraacetic acid and trypsin to produce a cell suspension and then centrifuged at 1500 rpm for 5 min. The concentrated cell suspension was collected in one tube and cell density was adjusted to 5×10^5 cells/mL by adding medium.

2.3. Mechanical stimulation device

A custom-made mechanical stimulation device was used to apply cyclic uniaxial strain to the cell–seeded constructs (Fig. 1a). The device included a component that applied mechanical stretching and a microcontroller that regulated the stepper motor driving the motion. The device included 3 separate wells, and each well was capable of containing up to 4 scaffolds in the culture medium. This unit allowed control over the frequency and amplitude of the strain applied to the scaffolds, and timers could introduce automatic rest periods in the cycle. The instrument was designed to fit inside a standard cell culture incubator.

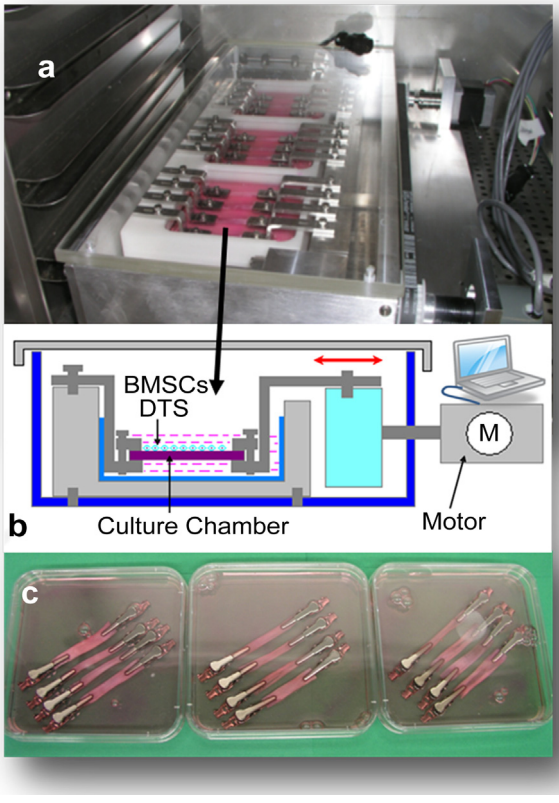

Fig. 1. A custom mechanical stimulation device. Upper panel, Photograph showing the inside of the incubator and mechanical stimulation device. Up to 12 cell-slice constructs (4 constructs in each of 3 dishes) are connected with a motor-driven frame that generates force and displacement. A microcomputer controls the cyclic strain parameters (i.e., stretch amplitude, rate, frequency, and daily stretch time) during each session (a). Schematic diagram of a bioreactor unit that includes a cell-slice construct loaded between stainless steel grips within a dish (b). Unstrained composites were not subject to cyclic loading and served as controls (c).