Biomaterials 34 (2013) 2024–2037

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Engineered scaffold-free tendon tissue produced by tendon-derived stem cells☆

Ming Ni [a,b,1], Yun Feng Rui [a,c,1], Qi Tan [a], Yang Liu [a], Liang Liang Xu [a], Kai Ming Chan [a,d,e,***], Yan Wang [b,**], Gang Li [a,d,e,*]

[a] Department of Orthopaedics and Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China
[b] Department of Orthopaedics, The General Hospital of Chinese People's Liberation Army, Beijing 100853, China
[c] Department of Orthopaedics, Zhongda Hospital, School of Medicine, Southeast University, 87 Ding Jia Qiao, Nanjing, Jiangsu 210009, China
[d] The Hong Kong Jockey Club Sports Medicine and Health Sciences Centre, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China
[e] Program of Stem Cell and Regeneration, School of Biomedical Science, and Li Ka Shing Institute of Health Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China

ARTICLE INFO

Article history:
Received 14 September 2012
Accepted 26 November 2012
Available online 14 December 2012

Keywords:
Tendon-derived stem cells
Connective tissue growth factor
Tenogenic differentiation
Scaffold-free
Cell sheet
Tendon tissue engineering

ABSTRACT

Most of the exogenous biomaterials for tendon repair have limitations including lower capacity for inducing cell proliferation and differentiation, poorer biocompatibility and remodeling potentials. To avoid these shortcomings, we intend to construct an engineered tendon by stem cells and growth factors without exogenous scaffolds. In this study, we produced an engineered scaffold-free tendon tissue (ESFTT) in vitro and investigated its potentials for neo-tendon formation and promoting tendon healing in vivo. The ESFTT, produced via tendon-derived stem cells (TDSCs) by treatment of connective tissue growth factor (CTGF) and ascorbic acid in vitro, was characterized by histology, qRT-PCR and immunohistochemistry methods. After ESFTT implanted into the nude mouse, the in vivo fluorescence imaging, histology and immunohistochemistry examinations showed neo-tendon formation. In a rat patellar tendon window injury model, the histology, immunohistochemistry and biomechanical testing data indicated ESFTT could significantly promote tendon healing. In conclusion, this is a proof-of-concept study demonstrating that ESFTT could be a potentially new approach for tendon repair and regeneration.

1. Introduction

Tendon injuries are common in both the workplace and sport activities with more than 30 million injuries occurred annually worldwide [1]. There were about 200,000 tendon and ligament repair surgeries performed annually in the USA [2]. The reported incidence of acute Achilles tendon injury was 18 per 100,000 people [3]; and these injuries typically occurred between the ages of 20 and 50 years [4]. Rotator cuff injuries are among the most common traumatic tears, with over 50,000 rotator cuff repair surgeries performed each year in the USA [5]. These injuries are difficult to manage because tendons do not heal by a regenerative process but via formation of a fibrotic scar, with poor tissue quality and mechanical properties and frequently result in long-term pain, discomfort and disability [6]. Tendon healing occurs in three overlapping phases. In the initial inflammatory phase, inflammatory cells enter the site of injury, chemotactic factors and inflammatory cytokines are released with increased vascular permeability, initiation of angiogenesis, stimulation of tenocyte proliferation. In the repair phase, tenocytes gradually migrate to the wound, and type III collagen synthesis is initiated and peaks during this phase, which lasts for a few weeks. After approximately 4–6 weeks, the modeling phase commences, the healing tissue is reshaped with decrease in cellularity and the type III collagens are being replaced by type I collagen and other tendon related ECM proteins during the remodeling phase. The inability of tendons to self-repair and the general inefficiencies of current treatments have spurred a demand for the development of tissue-engineering strategies for tendon repair and regeneration [7].

Most of the exogenous biomaterials for tendon repair have limitations including lower capacity for inducing cell proliferation and differentiation, poorer biocompatibility and remodeling potentials. Many challenges exist in tendon tissue engineering,

☆ This study has been accepted as podium presentation in the ORS 2012 Annual Meeting in San Francisco, California, and also was the winner of "Webster Jee Young Investigator Award" at 2012 ORS-ICHTS membership meeting from the International Chinese Hard Tissue Society.

* Corresponding author. Department of Orthopaedics and Traumatology, Faculty of Medicine, The Chinese University Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, PR China. Tel.: +852 3763 6153; fax: +852 2646 3020.
** Corresponding author. Tel.: +86 10 6693 9439; fax: +86 10 8821 9862.
*** Corresponding author. Department of Orthopaedics and Traumatology, Faculty of Medicine, The Chinese University Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, PR China. Tel.: +852 2636 2728; fax: +852 2646 3020.
E-mail addresses: kmchan@cuhk.edu.hk (K.M. Chan), yanwang301@yahoo.com (Y. Wang), gangli@cuhk.edu.hk (G. Li).
[1] Ni M and Rui YF are co-first authors with equal contribution in this work.

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2012.11.046

such as low cell proliferation and differentiation efficiency within exogenous biomaterials, unmatched rates between exogenous scaffold degradation and cell proliferation as well as extracellular matrix production, and different biomechanical properties compared with intact tendon. With the development of cell sheet-based bioengineering concepts, cell sheet engineering can provide natural cellular junctions, extracellular matrix and microenvironments [8–12]. The current investigation intends to construct an engineered tendon by stem cells and growth factors without exogenous scaffolds.

Tendon stem/progenitor cells, as a new cell type, was firstly identified in both human and mouse tendon tissues in 2007 [13], and we also isolated and characterized this unique stem cell population from rat tendon tissues [14,15]. We have found that tendon-derived stem cells (TDSCs) have higher mRNA expression of tenomodulin (Tnmd), scleraxis (Scx), type I collagen (Col1A1), decorin (Dcn) and biglycan (Bgn) than that of bone marrow derived mesenchymal stem cells (BMSCs) [16]. Our recent study further demonstrated TDSCs could promote earlier and better recovery after tendon injury as a new cell source for tendon repair [17]. However, higher mRNA expression of osteogenic markers such as alkaline phosphatase (ALP) and osteocalcin and chondrogenic markers aggrecan (Acan) and type II collagen (Col2A1) was also detected in the TDSCs comparing with that of BMSCs in vitro [16], indicating that TDSCs may also have higher osteo-chondrogenic potentials. The ectopic bone formation in tendon after transplantation of MSCs is an unwanted side effect that had been reported [18,19]. To avoid this potential risk, we propose that tenogenic differentiation of TDSCs in vitro may promote tendon healing and alleviate the risk of complication such as ectopic bone formation after transplantation of tenogenic TDSCs in vivo.

Connective tissue growth factor (CTGF) is a cystein rich protein (Cyr61), and nephroblastoma overexpressed gene family growth factor that can promote fibroblast proliferation and matrix formation in vitro [20]. The CTGF knockout mice lead to abnormal skeletal growth with impaired chondrocyte proliferation, angiogenesis, extracellular matrix production and turnover [21]. CTGF mRNA was highly expressed during early tendon healing in a chicken flexor digitiorum profundus tendon injury model [22], which implied that CTGF might be involved in tendon repair. Treatment of human BMSCs with CTGF and ascorbic acid was reported to induce fibroblastic differentiation with increased production and mRNA expression of collagen type I and tenascin C but not osteogenic, chondrogenic and adipogenic differentiation [23,24].

Ascorbic acid, as one form of vitamin C, plays an important role in the collagen and other extracellular matrixes (ECM) production [25–27], as well as to mimic the in vivo biological microenvironment of MSCs and regulate their proliferation and differentiation [28,29]. The intraperitoneal injection of vitamin C once for every 2 days accelerated the Achilles tendon healing with early angiogenesis and increased collagen synthesis in rat model [30]. Recently, Wei et al. reported that Vitamin C alone could promote MSCs sheet formation and tissue regeneration by elevating telomerase activity [12].

In this study, we would like to test the production of engineered scaffold-free tendon tissue (ESFTT) in vitro via tenogenic differentiation of TDSCs through treatment of CTGF and ascorbic acid; and further test the hypothesis of using the ESFTT to promote tendon repair in a rat patellar tendon window injury model.

2. Materials and methods

2.1. Isolation and culture of rat GFP-TDSCs

All experiments were approved by the Animal Research Ethics Committee, the Chinese University of Hong Kong. 4-6-week-old male GFP (Green Fluorescent Protein) Sprague–Dawley rats, weighting 250–300 g were used in this study. The procedure of isolation and culture rat GFP-TDSCs was established in our previous work as illustrated in Fig. 1A [14,15]. In brief, the patellar tendons were excised from healthy rats overdosed with 2.5% sodium phenobarbital. The tissues were minced, digested with type I collagenase (3 mg/ml; Sigma–Aldrich, St Louis, MO, USA) and passed through a 70 μm cell strainer (Becton Dickinson, Franklin Lakes, USA) to yield single-cell suspension. The released cells were washed in PBS and resuspended in low glucose Dulbecco's Modified Eagle Medium (LG-DMEM) (Invitrogen Corporation, Carlsbad, USA), 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine (complete culture medium) (all from Invitrogen Corporation, Carlsbad, USA). The isolated cells were plated at low density (500 cells/cm^2) and cultured at 37 °C, 5% CO_2 to form colonies. At day 2 after initial

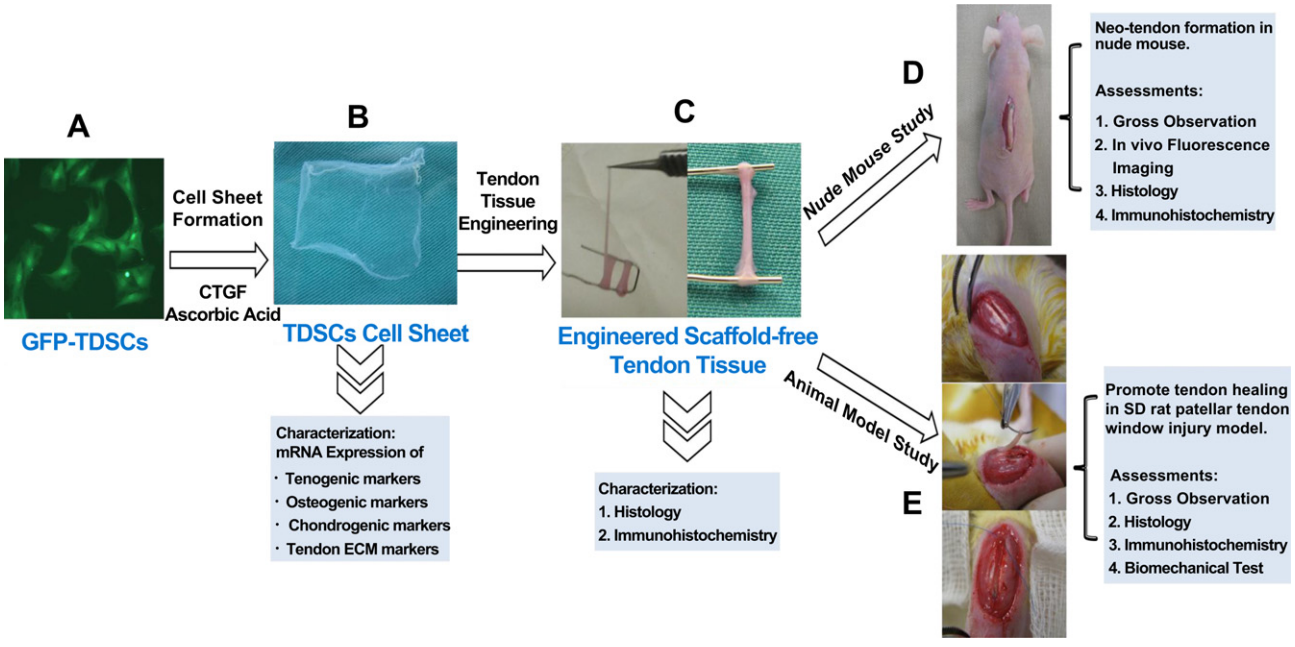

Fig. 1. The GFP-TDSCs (A) formed a cellular sheet (B) after treated by CTGF and ascorbic acid. The TDSCs cell sheet (B) was then rolled up and loaded on a 1 cm wide U-shaped spring to form engineered scaffold-free tendon tissue (C), which was sutured on the back of nude mice (D) to form neo-tendon in vivo. The engineered scaffold-free tendon tissue was sutured to the patellar bone and tibia tuberosity to promote tendon healing in a SD rat patellar tendon window injury model (E).