Growth Hormone & IGF Research xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Growth Hormone & IGF Research

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

Insulin-like growth factor I enhances collagen synthesis in engineered human tendon tissue

Andreas Herchenhan *, Monika L. Bayer, Pernilla Eliasson, S. Peter Magnusson, Michael Kjaer

Institute of Sports Medicine Copenhagen, Bispebjerg Hospital, Bispebjerg Bakke 23, Building 8, 2nd floor, 2400 Copenhagen, Denmark
Centre for Healthy Aging, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 9 May 2014
Received in revised form 6 August 2014
Accepted 11 September 2014
Available online xxxx

Keywords:
Tendon formation
Fetal bovine serum
Fibrillogenesis
Tenomodulin
Scleraxis
Collagen content
3D cell culture

ABSTRACT

Objective: Isolated human tendon cells form 3D tendon constructs that demonstrate collagen fibrillogenesis and feature structural similarities to tendon when cultured under tensile load. The exact role of circulating growth factors for collagen formation in tendon is sparsely examined. We investigated the influence of insulin-like growth factor I (IGF-I) on tendon construct formation in 3D cell culture.
Design: Tendon constructs were grown in 0.5 or 10% FBS with or without IGF-I (250 mg/ml) supplementation. Collagen content (fluorometric), mRNA levels (PCR) and fibril diameter (transmission electron microscopy) were determined at 7, 10, 14, 21 and 28 days.
Results: IGF-I revealed a stimulating effect on fibril diameter (up to day 21), mRNA for collagen (to day 28), tenomodulin (to day 28) and scleraxis (at days 10 and 14), and on overall collagen content. 10% FBS diminished the development of fibril diameter (day 14), collagen content (at days 21 and 28) and mRNA expression for collagen, tenomodulin and scleraxis.
Conclusion: IGF-I supplementation promotes early onset of tensile load induced collagen formation and tendon structural arrangement, whereas the FBS concentration routinely used in cultures diminishes collagen expression, collagen content and fibril formation.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

The connective tissue of tendon is responsible for efficient force transmission between muscle and bone and has therefore exceptional mechanical properties. Tendon is structurally arranged in a highly hierarchical manner ranging from single collagen molecules to the entire tendon unit [1]. Collagen is the most abundant protein in tendon, and it provides the basis for the structural and mechanical integrity of the tissue. Collagen development and structural organization are crucial processes during embryogenesis and are essential given the relatively low turnover of tendon collagen tissue in adult life [2].

The *in vivo* environment of cells is a three-dimensional (3D) extracellular matrix (ECM); however, most *in vitro* studies to date utilize cells in 2D monolayers [3,4]. More recently, 3D cell culture approaches have shown considerable advantages over 2D cell monolayers. Fibroblast adhesion to substrate was shown in 3D to be improved six-fold over 2D; cell migration is significantly increased and 3D-specific cell adhesions are established [5]. The 3D cell shape and the available space can even decide upon cell fate, while cells in 2D are limited to a flat arrangement [6].

Fibrin gels are suggested to serve as a biocompatible, stable and bioactive microenvironment for successful 3D cell culture [7,8]. We recently introduced a 3D tendon construct system from adult human tendon fibroblasts [9,10]. Driven by their actin-myosin machinery, the fibroblasts contract a fibrin gel, which is constrained by two opposed anchor points, leading to a rod-shaped structure [11]. The cells are thereby aligned along the longitudinal axis, similar to their in vivo state [10, 11]. Embryonic-like cell protrusions—earlier termed fibripositors—are established as a link between the cells and the collagen network, and the construct shows has viscoelastic mechanical properties under tensile testing [9,11]. Finally, the tendon constructs develop larger collagen fibrils with increasing diameter during 5 weeks that result in increasing resistance toward tensile mechanical forces [10]. Taken together, these characteristics prove that the tendon construct is a valid model for studying tendon development. Further studies have shown that the system is sensitive to stretching stimuli, which leads to increased collagen expression and fibril diameter [12,13].

The mean collagen fibril diameter and the diameter distribution are characteristics that vary considerably between tissues depending on their function. Ligaments such as the anterior cruciate ligament have a narrow spectrum with rather low diameter fibrils (up to 100 nm), while load-bearing tendons like the hamstring tendon show a broad

* Corresponding author at: Institute of Sports Medicine Copenhagen, Bispebjerg Hospital, Bispebjerg Hospital, Bispebjerg Bakke 23, Building 8, 2nd floor, 2400 Copenhagen, Denmark. Tel.: +45 3531 6091.
E-mail address: andreashe@sund.ku.dk (A. Herchenhan).

http://dx.doi.org/10.1016/j.ghir.2014.09.001
1096-6374/© 2014 Elsevier Ltd. All rights reserved.

Please cite this article as: A. Herchenhan, et al., Insulin-like growth factor I enhances collagen synthesis in engineered human tendon tissue, Growth Horm. IGF Res. (2014), http://dx.doi.org/10.1016/j.ghir.2014.09.001

spectrum with diameters reaching over 200 nm [14]. Adult tendon typically show a bimodal collagen fibril diameter distribution with one peak at low diameters of approximately 40 nm and a second peak around 140 nm [15,16].

Tendon cells experience continuous mechanical strain that has been proven to be vital for tendon development [12,17–20]. In addition, growth-stimulating signals can be elicited by local accumulation of growth factors such as Insulin-like Growth Factor-I (IGF-I) [3,4,21]. It has been demonstrated *in vivo* that local injection of IGF-I into the patellar tendon of humans yields an upregulation of matrix related genes such as collagen type I [22,23]. IGF-I has been shown to increase cell proliferation [4] and to promote matrix formation [3]. Furthermore, IGF-I inhibits cell death in isolated tendon cells by activating the protein kinase B signaling pathway [24]. Although these *in vitro* approaches using isolated tendon cells in 2D cultures have provided major insight into our understanding of tendon growth and development, it may not entirely represent the physiological responses *in vivo*. We aimed to study the effect of IGF-I on collagen development in the 3D tendon construct system *in vitro*, in which the cells are arranged more similar to their *in vivo* configuration [9,10,12,17,25].

In addition to growth factors, other substances in the cell culture medium used for the development of a tendon construct may affect the outcome. Fetal bovine serum (FBS) is routinely used for cell culture systems since it provides vital components for cells in culture, including growth factors, proteins, vitamins and hormones [26]. However, the detailed composition of FBS is difficult to determine and varies between different batches [27,28]. Surprisingly little attention has been given to the potential effect of the FBS concentration upon cell reactions in culture systems, and the fact that growth factor concentrations vary depending on the donor animal [29].

The aim of this study was to investigate the effect of IGF-I and FBS concentration upon tenocytes in a 3D cell culture. A very low level of FBS supplementation was chosen in order to pinpoint the effect of IGF-I on tendon constructs and collagen fibrillogenesis in absence of interacting factors that FBS contains. Therefore, tendon constructs were treated either with a standard dose of 10% FBS or a very low dose of 0.5%, with or without 250 ng/ml IGF-I. Construct development was monitored up to 4 weeks after seeding by means of construct ultrastructure, collagen fibril diameter, total collagen content and gene expression.

2. Materials and methods

2.1. Cell culture

Tendon fibroblasts were isolated from human semitendinosus and gracilis tendon as previously described in detail [9]. Briefly, patients (18–32 years old) undergoing reconstructive surgery after anterior cruciate ligament (ACL) rupture gave their informed consent to donate excess tendon tissue. The tissue was transported to a cell culture laboratory immediately following harvest. Under aseptic conditions, the tissue was minced into pieces ~2 mm^3 and digested overnight in DMEM/F12 (Gibco, Invitrogen) supplemented with 0.1% collagenase type II (Worthington) and 20% fetal bovine serum (FBS) (Gibco, Invitrogen). Following repeated washes in culture medium (DMEM/F12, 10% FBS), the cells were seeded into flasks and cultured until the next passage. Cells from at least five different donors in the 2nd to 6th passage were used for experiments. The present experiment was approved by the local ethical committee (ref. H-3-2010-070).

2.2. Tendon construct formation

Tendon constructs from human tenocytes were assembled as described previously with minor modifications [9]. Briefly, each well of a six-well plate was coated with ~1.5 ml SYLGARD (Dow-Chemicals) and allowed to set at 55 °C for 48 h. Next, two short silk sutures (0.5 cm, Ethicon) were pinned onto the coated plates with minutien insect pins (0.1 mm diameter) (Fine Science Tools GmbH) with a distance of 1.5 cm in between sutures. The plates were sterilized by immersion in 70% ethanol for 45 min. Human tendon fibroblasts were suspended in culture medium containing 4 mg human fibrinogen, 10 μg/ml aprotenin and 1 unit of human thrombin (all Sigma Aldrich) to a final concentration of 2.5×10^5 per 800 μl and rapidly spread over the complete surface of the coated wells. The cell embedded fibrin gel was allowed to set for 30 min at 37 °C, covered with medium and cultured until the matrix was fully contracted between the anchor points. Every other day, culture medium supplemented with 0.2 mM L-ascorbic acid 2-phosphate and 0.05 mM L-Proline (Sigma Aldrich) was replaced and adhesions to the side of the well were detached using a fine pipette tip to allow gel contraction. The constructs were divided into four treatment groups: DMEM/F12 medium supplemented with (1) 0.5% FBS, (2) 0.5% FBS + 250 ng/ml IGF-I, (3) 10% FBS and (4) 10% FBS + 250 ng/ml IGF-I. A first batch of constructs was analyzed at 21 and 28 days post seeding, where we could see an effect of IGF-I (denoted as first study part). In order to see the onset of the IGF-I effect, more constructs were grown with 0.5% FBS with or without IGF-I and analyzed at 7, 10 and 14 days, respectively, which were analyzed for collagen content and gene expression and are presented as results of the second part of the study.

2.3. Transmission electron microscopy

After discarding culture medium and rinsing in PBS, constructs were fixed in 2% Glutaraldehyde in 0.05 M phosphate buffer for 30 min at RT. The constructs were then cut in three equal pieces and fixed in fresh fixative for at least 2 h at 4 °C. After washing in 0.15 M phosphate buffer, the samples were postfixed with 1% OsO_4 in 0.12 M sodium cacodylate buffer for 2 h at room temperature. Following another washing in dH_2O, the samples were stained en bloc with 1% aqueous uranyl for 16 h at 4 °C, dehydrated in a graded serious of ethanol and embedded in Epon (Hexicon, Houston, Texas, USA) according to standard procedures.

Ultrathin cross sections were cut with a Reichert-Jung Ultracut E microtome using a diamond knife and were collected on one-hole copper grids with Formvar supporting membranes. Images were acquired in a Philips TM 100 transmission electron microscope, operated at an accelerating voltage of 80 kV, with a Megaview 2 camera and processed with the iTEM AnalySIS software package (ResAlta Research Technologies, Golden, USA). The NIH-based image-processing program, Image J, was used for measurement of collagen fibril diameters. On randomly selected micrographs, 300 fibrils were analyzed per specimen, cell line, treatment and time point, respectively.

2.4. Quantitative real-time PCR

The amount of mRNA for target genes was measured using quantitative real-time reverse transcriptase (RT) PCR. An overview over targets and primers sequences is provided in Table 1. First, tendon constructs were harvested and transferred to RNAse free tubes containing 1 ml TriReagent (Molecular Research Centre, Cincinnati, OH, USA), 5 stainless steel beads of 2.3 mm in diameter and 5 silicon-carbide sharp particles of 1 mm for mechanical disruption (BioSpec Products, Inc., Bartlesville, Oklahoma, USA). For RNA isolation, samples were mechanically

Table 1
PCR primer List.

mRNA	Sense	Antisense
RPLP0	GGAAACTCTGCATTCTCGCTTCCT	CCAGGACTCGTTTGTACCCGTTG
COL1A1	GGCAACAGCCGCTTCACCTAC	GCGGGAGGACTTGGTGGTTTT
COL3A1	CACGGAAACACTGGTGGACAGATT	ATGCCAGCTGCACATCAAGGAC
Scx	CAGCCCAAACAGATCTGCACCTT	CTGTCTTTCTGTCGCGGTCCTT
Tnm	GAAGCGGAAATGGCACTGATGA	TGAAGACCCACGAAGTAGATGCCA

Please cite this article as: A. Herchenhan, et al., Insulin-like growth factor I enhances collagen synthesis in engineered human tendon tissue, Growth Horm. IGF Res. (2014), http://dx.doi.org/10.1016/j.ghir.2014.09.001

Download English Version:

https://daneshyari.com/en/article/5901810

Download Persian Version:

https://daneshyari.com/article/5901810

Daneshyari.com