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REVIEW ARTICLE

Incorporating Connective Tissue Growth Factor into Regenerative and Personalised Medicine for Tendon and Ligament Regeneration: A Systematic Review

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ABSTRACT

Background: Tendon and ligament disorders particularly those of the weight bearing joints are associated with decreased physical activities affecting the quality of life. Current management includes conservative and surgical approaches requires a relatively long recovery time, in addition to the post-operative complications such as infection of the wound site and joint stiffness if managed surgically. Connective tissue growth factor is a type of growth factor expressed during second phase of the healing process and is responsible for tissue healing by stimulating the release of extracellular matrix responsible for tissue healing. Hence, the objective of this review is to identify available studies relating to the used of connective tissue growth factor in tissue culture for tendon and ligament healing.

Methods: Studies were identified from PubMed Central, BioMed Central, ScienceDirect, and Wiley Online Library with the keywords: Connective Tissue Growth Factor 'OR' CTGF 'OR' CCN 'AND' Regeneration 'AND' Healing from the year 2019 to present, 2024. All literatures were reviewed in three phases by two reviewers.

Results and Conclusion: There were only three articles which met our inclusion criteria. In general, connective tissue growth factor had been reported to be beneficial for ligament and tendon regeneration. However, the effective connective tissue growth factor dose for optimal ligament and tendon regeneration healing could not be determined due to the different cell source and delivery methods utilised among the identified articles.

Introduction

Tendon and ligament disorders particularly those of the weight bearing joints are associated with decreased physical activities affecting the quality of life [1–4]. Current management includes conservative and surgical approaches [5]. However, the recovering time required is relatively long in addition to the post-operative complications such as infection of the wound site and joint stiffness if managed surgically [6]. Given these, regenerative and personalized medicine had been widely studied and was reported to yield positive effect in the regeneration and recovery of various tissues of the human body [7–9]. The administration of regenerative and personalized medicine includes the use of cellular exosomes, cellular therapy where cells are injected locally onto the site of injury or systemically, growth factors, which being administered through control release scaffolds, or tissue engineering [10–15].

Connective tissue growth factor (CTGF) was first discovered as a polypeptide growth factor secreted by human endothelial cells that plays an important role in stimulating chemotaxis and DNA synthesis of fibroblasts [16]. Subsequent studies also reported where CTGF promotes proliferation, migration, and adhesion of fibroblasts as well as extracellular matrix formation and remodelling that occurs in the normal physiological process of tissue healing [17–20]. While collagens are the primary building block for most tissues in the human body, the study by Dorn et al. (2018) had reported where CTGF involves in the activation of fibroblast and production of collagen I and III in vitro [21]. We believed that CTGF preconditioned cells could improve collagen I and III secretion which could improve tendon and ligament healing. Hence, the objective of this review is to identify available studies relating to the used of CTGF in tissue culture for tendon and ligament healing.

Methodology

LITERATURE REVIEW

We performed a comprehensive search of journals in the PubMed Central, BioMed Central, ScienceDirect, and Wiley Online Library to identify related studies regarding to CTGF in tendon and ligament regeneration. The keywords used were Connective Tissue Growth Factor 'OR' CTGF 'OR' CCN 'AND' Regeneration 'AND' Healing from the year 2019 to present, 2024.

SELECTION OF RESEARCH ARTICLES

The selection of articles included original research related to CTGF in tendon and ligament regeneration done *in vivo* or *in vitro*. The excluding criteria for this study include any form of review articles such as narrative or systematic review, news, letters, editorials, case studies or studies that do not link CTGF to tendon or ligament regeneration, studies that focus on natural products, scaffold, and cancer related studies. The reason where articles pertaining to natural products, and scaffolding were excluded which is due to the reasons where these products may contain other ingredients or substances which could serve as a confounding factor that affect the tissue healing process.

DATA EXTRACTION AND MANAGEMENT

Works of literature were reviewed in three phases (Figure 1). Articles were selected based on the title (first phase) and been screened based on the abstracts (second phase). Those that did not meet the inclusive criteria were excluded. Finally, the full text of the selected articles was reviewed before being reported. All papers were read thoroughly to exclude literature that did not meet our inclusion criteria.

To minimize biases, a single blinded review method was adopted where the two reviewers assigned to review the papers from the first to the third phase were not supposed to discuss their findings with each other during the review process. The differences in the agreement after the final review were resolved by discussion between the reviewers. The works of literature included in this study were the results from a mutual agreement by both reviewers.

To standardise our data collection, all data extraction was carried out independently using a standard data collection form. In brief, the following data was recorded from the works of literature: (1) author(s) name and year along with references; (2) study design; (3) types of cells used; (4) type(s) of ligaments and/or tendon studied; (5) brief description; and (6) study outcomes.

Results

The search led to the retrieval of 76 articles consisting of 50, 6, 18 and 2 articles in PubMed Central, BioMed Central, ScienceDirect and Wiley Online Library respectively (Figure 2). Duplicated publications were excluded. During the initial title screening phase, a total of 25 articles (8, 9, 6 and 2 articles from PubMed Central, BioMed Central, ScienceDirect and Wiley Online Library respectively) were excluded from the study due to its non-compliance with the inclusion criteria of this study. Upon abstract screening, only 19 articles met our inclusion criteria and only three articles were included after the final full text screening. Upon deliberation, by the two reviewers involved in this study, it is also decided where studied pertaining to cancer research and natural supplements should be excluded as the aim for this study is based on the regenerative potential of CTGF cultured cells. While regeneration and cancer share the similar genetic mechanisms and cellular processes, the regenerative process in cancer research are usually incomplete as such improve proliferation does not always mean improved regeneration. Also, studies related to the used of scaffold and natural supplements were excluded due to the presence of other active ingredients which may serve as a confounding factor. Hence, the selection of articles was based on mutual agreement by the two reviewers and only three articles met our inclusion criteria and were included in present review.

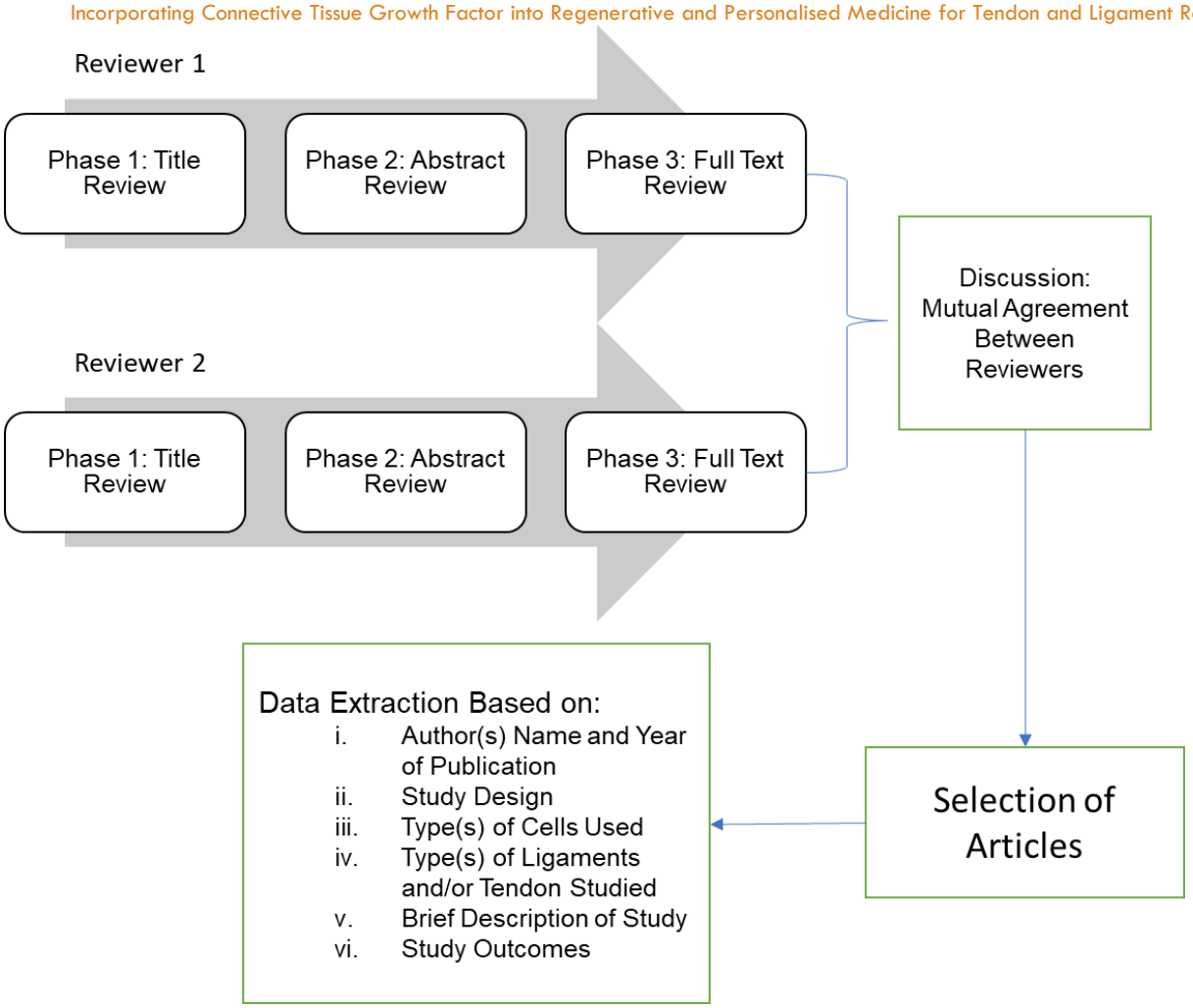

Figure 1: Flow chart on the article selection process

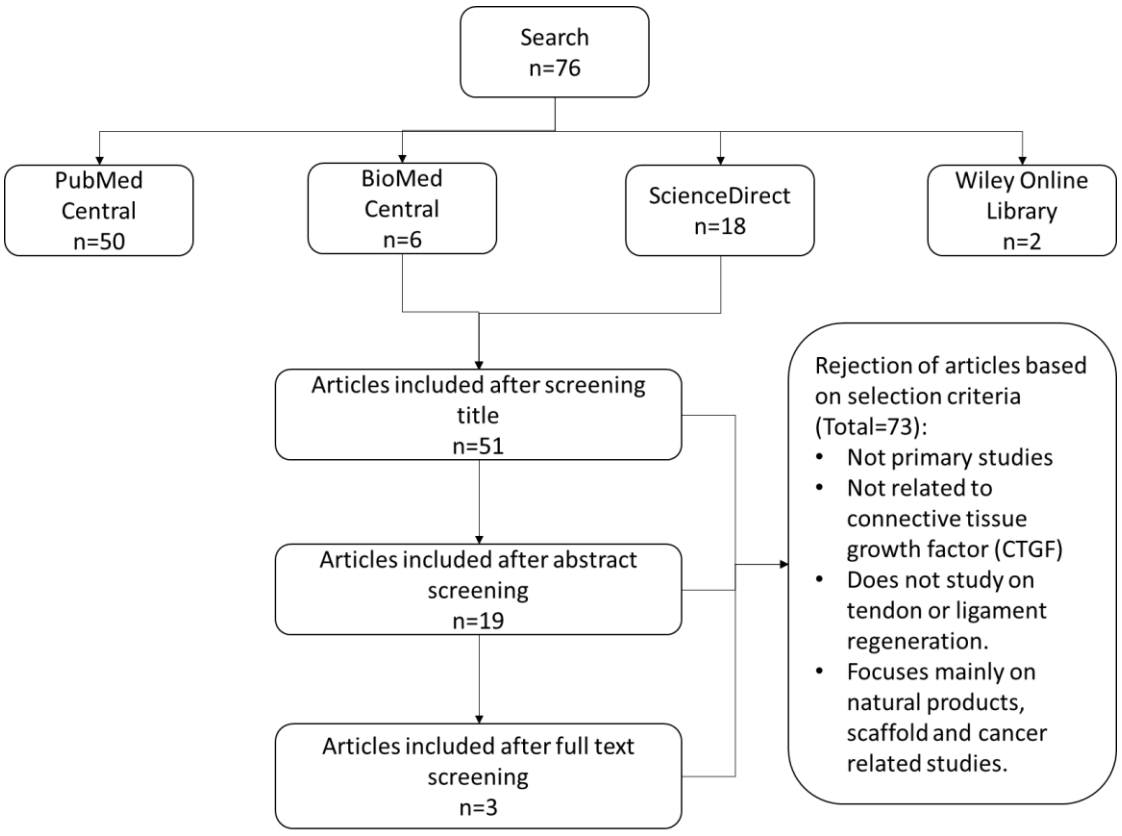

Figure 2: Article distribution

Table

Authors, Year	Cell type	Comparisons And Dosage	Duration	Outcome Measures	Findings
Shen H, Tarafder S, Park G, et al. (2022) [23]	Cells from canine flexor digitorum profundus tendon fragments	1. Oxo-M (1 mM) and 4-PPBP (10 μM) 2. CTGF (100 ng/mL) 3. Control	1 week	quantitative RT-PCR for tendon-related mRNA markers: • COL-I • COL-III • TN-C • VIM • TNM • SCX	CTGF mimics stimulate proliferation, tenogenesis, and tendon-specific extracellular matrix synthesis, indicative of improved tendon healing.
Li X, Pongkitwitoon S, Lu H, Lee C, Gelberman R, Thomopoulos S. (2019) [24]	Passage 2 Adipose-derived stem cells (ASCs)	0, 1, 10, or 100 ng/ml CTGF	14 days	• Real Time PCR (gene expression) • Westen Blot (tenogenic markers expression) • Immunofluorescence Staining (Tenogenic protein expression) • CCK-8 assay (Cell viability) • Cignal 45-Pathway Reporter Arrays (Signalling Pathway Assessment)	• CTGF induced Tenogenic Differentiation of ASCs based on Real Time PCR and Western Blot. The most effective dose and treatment time for CTGF in this aspect was 100 ng/ml for 14 days. • CTGF treatment Induced Proliferation of ASCs with increased concentration to day 7 based on CCK-8 assay. • CTGF-Induced Tenogenesis and Proliferation of ASCs was Dependent on the ERK1/2 and FAK Pathway
Rui YF, Chen MH, Li YJ, et al. [25]	Aged tendon-derived stem/progenitor cells	CTGF-siRNA Transfection	72 hours	• Westen Blot (tenogenic markers expression) • Immunofluorescence Staining • β-Galactosidase Staining • CCK-8 assay (Cell viability) • Microarray Analysis • Quantitative RT-PCR • Cell Cycle Analysis • Colony-Forming Ability (CFA) Assays	• Recombinant CTGF treated cells possess senescence-delaying potential based on β-Galactosidase Staining • Recombinant CTGF treatment significantly increased the colony number and proliferative rate compared with aged tendon-derived stem/progenitor cells based on CCK-8 assay and Colony-Forming Ability (CFA) Assays. • Recombinant CTGF Increased the Tendon-Related Marker Expressions of aged tendon-derived stem/progenitor cells based on RT-PCR. • CTGF Regulates tendon-derived stem/progenitor cells Aging through Cell Cycle Progression.

Discussion

Studies on the role on CTGF culture cells in ligaments and tendon healing are relatively scarce. Present systematic review identified only three studies consisting of two in vitro and one in vivo study in regard to this area. There were no clinical trials identified and the available articles were considered the lowest level of reliable evidence according to the evidence pyramid [22]. All the identified studies reported favours CTGF preconditioned cells to have a great potential in enhancing the healing potential of the various cell uses via regenerative and personalised medicine approach in treating tendon injuries [23–25]. Although there were no studies identified on ligament healing, such findings suggested a promising research prospect on ligament and tendon healing using CTGF preconditioned mesenchymal stem cells. As such, tendon and ligaments are dense connective tissue that connects muscle to bone, and bone to bone respectively [26]. Hence, they share a more or less similar extracellular matrix components which consists of predominantly of type I collagen and a small amount of other collagens, including types III, IV, V and VI which is secreted by the residing cells [27]. Thus, this explains the use of tendon graft particularly the hamstring tendon to repair and substitute the torn anterior cruciate ligament (ACL) in the event of complete ACL tear injury [28]. With this, we decided to systematically review papers pertaining CTGF preconditioned cells in both ligament and tendon healing. To our knowledge, this is the first systematic review conducted on CTGF preconditioned cells in both ligament and tendon healing since the past years. The following sections summarise and discuss the role of CTGF in tendon and ligament healing based on existing studies and theories.

CTGF in Tissue Healing Mechanism

Ligament and tendon injuries accounts up to 50% of all musculoskeletal injuries [26]. In an event of a sprain ligament or strain tendon, the first response initiate by the human body is to maintain haemostasis by preventing blood loss through rapid contraction of the involved blood vessels and formation of blood clots [29,30]. Platelets, macrophages, cellular secretome and fibroblasts responsible for tissue healing travels through the adjacent blood vessels. Unfortunately, due to its poor vascularity, recovery from a sprain ligament or strain tendon is relatively time consuming [31]. Hence, affecting the quality of live as well as increase the time of from sports among athletes which greatly affect their sports performance. After maintaining haemostasis, inflammation sets in within few hours to a day which last for approximately two to three days where cell debris and necrotic tissues were removed by macrophages [32]. Up to this stage, mainly the transforming growth factor beta (TGF-β) and insulin growth factor-1 (IGF-1) were the main growth factors responsible for the events occurring during the haemostasis and inflammatory phase [9].

Connective tissue growth factor were elevated particularly during the phase II reparative/proliferative phase of the healing process [9]. It is a multifunctional protein which participates in a number of cellular events including proliferation, survival, adhesion and migration, differentiation, epithelial-mesenchymal transition, and formation of extracellular matrix [33]. Hence, this leads to our believes where the present of this growth factor is beneficial to wound and tissue healing. However, according to Fu Mingyang and colleagues (2022), CTGF contributes to the pathogenesis and progression of diseases such as inflammation, fibrosis, and cancer through a variety of disease-related pathways including the Hippo pathway, p53 and nuclear factor kappa-B (NF-κB) pathways. Also, there were number of studies which reported CTGF to promote fibrosis through prolonged collagen synthesis and deposition leading to keloid formation [34–37]. In the attempt to prevent such side effects, we proposed CTGF cultured cells to be used instead of local administration of CTGF into the injured site. We believed that these cells may temporarily processed increase proliferative and collagen secretion potential which could improves and speed up tissue healing. At the same time may act as a negative feedback mechanism, where the increase speed of healing reduced CTGF presence in the injured site thus, improving the speed of the healing process as well as the quality of scar. With such believes, a systematic review was conducted to identify available studies relating to the used of CTGF in tissue culture for tendon and ligament healing.

In fibrosis and cancer related studies, the presence of CTGF is believed to makes the condition worse by promoting the proliferation of fibroblasts, stellate cells, or carcinoma cells yet, this growth factor also exert an anti-proliferative potential on WiT49 cells, which is also a type of cancer cell lines [33,38]. In regenerative medicine, however, increase cellular proliferation, differentiation, and secretion of extracellular is important to replace the damaged structures while maintaining its original function [39]. Hence, the role of CTGF in cell culture will act as a stimulant to increase cellular proliferation. All articles included in present review reported where CTGF increases cellular proliferation. According to X. Li's et al. (2019) and Rui et al. (2019) CTGF stimulates proliferation in adipose stem cells and tendon-derived stem/progenitor cells respectively based on Cell Counting Kit-8 (CCK-8) assay to measure cell proliferation [24,25]. The study conducted by Shen and colleagues (2022) also reported CTGF to be associated with CD146, a cellular marker which is associated with increased cancer cell proliferation, motility, metastatic dissemination, and tumour angiogenesis [23].

Cellular differentiation is another important factor in tissue healing [40,41]. In the context of ankle and ligament injury, the administered cells should possess the ability to differentiate into tenocytes in treating muscle tears, and fibroblasts in treating ligament tears as according to their respective histological structure [5,25,42,43]. Failure might result in non-healing or abnormal growth on the site of injury if the cultured cells were tuned to differentiate into other structures. To date, we are still unable to determine the exact *in vitro* culture formulation that could guide the pre administered stem cells to differentiate into either tenocytes or ligament fibroblasts in addition to the presence of numerous conflicting evidence. However, it is believed that the *in vivo* environment in the injury site plays a major role in guiding the differentiation of the administered stem cells into the respective cells of the injured structure [44–46]. Additionally, hamstring tendon grafts were used for anterior cruciate ligament

reconstruction due to various factors including better success rate, less scarring on the operation site and faster return to range of motion [28]. Histologically, the graft will undergo a process known as ligamentization where the transplanted tendon will undergo phases including necrosis, revascularization, and remodelling which gradually shape it to resemble the original ligament tissue in structure and function over a period of 12 to 18 months [47]. Also, many articles generalised the intervention of cellular therapy and secretome to both ligaments and tendon with the use of stem cell-conditioned medium [48–50], although they share the similar molecular, cellular, and hierarchical structure and are elastic tissues that contribute to joint movement but different composition [51,52]. Hence, due to limitation in the available articles pertaining to this aspect, we decided to include articles related to the used of CTGF preconditioned cells in both ligaments and tendon healing. Based on the two identified articles, CTGF had been reported to induced tenogenesis and similarly, the other article reported to upregulate tendon-related marker expressions of aged tendon-derived stem/progenitor cells based on RT-PCR [23–25]. However, previous study in Lee and colleague's laboratory on rats calvaria reported where CTGF attenuate the ability of mesenchymal stem cells to differentiate into osteogenic, chondrogenic and adipogenic lineages [19]. Therefore, prompt the believed where cellular differentiation regardless on the presence of CTGF are strongly dependant on the *in vivo* environment.

Another important factor in ligament and tendon healing is the ability to secrete extracellular component to replace the damaged structure. In this aspect, there were some slight differences between both ligaments and tendons. As such, the study by Kouroupis et al (2014) reported were the water content in ligaments accounted approximately 65 to 70% of its wet weight while only 55% in tendons [52]. Collagen consists of 70 to 80% of ligaments and approximately 85% of tendon's dry weight with type I collagen being the most abundant collagen subtype among all the other subtypes of collagen present [6]. Thus, present study evaluates on collagen I secretion by cells induced by CTGF. All articles included in present review reported CTGF to increase the expression of collagen I and collagen III mRNA based on quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) analysis. Despite that, there were limited data on collagen I and III secretion in CTGF preconditioned cells. Moreover, apart from tissue healing, the increase in collagen secretion had been largely reported to be involved in fibrotic changes [53–57]. While fibrotic scarring is a crucial part of the healing process, it can be detrimental especially during excessive scarring where the normal function of the ligaments and/or tendon were disrupted [58]. Also, there were no articles identified to date which studies on the effective CTGF dose for optimal healing.

Limitations and future suggestions

While present review provides preliminary information on the role of CTGF in ligament and tendon healing, we are unable to determine the effective dose for optimal healing. Additionally, the available data were too limited for a meta-analysis. Since, CTGF is one on the major growth factors involved in tissue healing and the scarcity of available studies, there are huge potential in this field of study including (1) CTGF culture and administration methods (2) the effective dose of CTGF on different cell types and sources in treating tendons, and ligaments obtained from different parts of the body, and (3) the safety dose, complications and side effects that might arise in such area.

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