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Review

Techniques for biological characterization of tissue-engineered tendon and ligament

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Abstract

Injuries to tendons and ligaments are prevalent and result in a significant decrease in quality of patient life. Tissue-engineering strategies hold promise as alternatives to current treatments for these injuries, which often fail to fully restore proper joint biomechanics and produce significant donor site morbidity. Commonly, tissue engineering involves the use of a three-dimensional scaffold seeded with cells that can be directed to form tendon/ligament tissue. When determining the success of such approaches, the viability and proliferation of the cells in the construct, as well as extracellular matrix production and structure should be taken into account. Histology and histochemistry, microscopy, colorimetric assays, and real-time reverse transcriptase-polymerase chain reaction (RT-PCR) are techniques that are employed to assess these biological characteristics. This review provides an overview of each of these methods, including specific examples of how they have been used in evaluation of tissue-engineered tendon and ligament tissue. Basic physical principles underlying each method and advantages and disadvantages of the various techniques are summarized.

Keywords: Tendon tissue engineering; Ligament tissue engineering; Biomaterial scaffold; Characterization techniques

Contents

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1. Introduction

Over 800,000 people seek medical attention each year for injuries to ligaments, tendons, or the joint capsule [1]. Ruptures and other damage to tendons and ligaments can cause great pain and decrease the functionality of the joint complex. There are numerous areas throughout the body where tendons and ligaments experience such injuries. These include the patellar tendon [2], the anterior cruciate ligament [3], the posterior cruciate ligament [4], and the medial collateral ligament [5] in the knee; the Achilles tendon [6] at the heel; the anterior talofibular ligament [7] and the calcaneofibular ligament [8] in the ankle; the radial ulnohumeral ligament [9] and the lateral ulnar collateral ligament [10] in the elbow; the digital flexor tendon [3,6] and the ulnar collateral ligament [11] in the hand; the scapholunate ligament [12] in the wrist; the rotator cuff tendons [3], the acromioclavicular ligament [13], the coracoclavicular ligament [14], and the coracohumeral ligament [15] at the shoulder; and the gluteus medius tendon [16] at the hip.

Often, natural healing of these injuries is insufficient since many tendons and ligaments possess a limited capacity to regenerate [6,17]. While certain tendons and ligaments can be repaired by suturing the injured tissue back together, some heal poorly in response to this type of surgery, so the use of grafts is required [18]. Unfortunately, finding suitable graft material can be problematic. Autografts from the patient may result in donor site morbidity, while allografts from cadavers may cause a harmful response from the immune system and are limited in supply [19]. In both cases, the graft often does not match the strength of undamaged tissue [19].

Such shortcomings in repair of tendons and ligaments may be overcome through tissue-engineering approaches. Tissue-engineering strategies involve the use of a biomaterial carrier, cells, and/or bioactive factors to promote tissue regeneration via natural processes in the body [20]. One common tissue-engineering approach involves using a three-dimensional (3D) scaffold to direct pre-seeded cells to create viable tendon/ligament tissue [21–25]. In order to determine the relative success between different tissue-engineering techniques, specific outcome measures (success criteria) are required. Biological assessment, in conjunction with other tests, is a crucial part of the optimization and final clinical application of tissue-engineered tendons and ligaments, and, thus, is the subject of this review. A variety of techniques exist to characterize biological components of tendon and ligament tissue, but only a subset of these methods has been employed to evaluate tissue-engineered tendons and ligaments. Therefore, this review focuses on those most commonly used to assess biological parameters of tissue-engineered tendon/ligament constructs.

2. Function and structure of tendon and ligament tissue

Biological evaluation is carried out on tissue-engineered constructs to determine to what extent the construct tissue replicates the structure and chemical composition of native tendon/ligament tissue. Thus, a brief review of the structure and function of tendons and ligaments is included in this section.

2.1. Function of tendon and ligament tissue

Tendons and ligaments are dense, regularly arranged, connective tissues that induce or guide joint movement [26].