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Strategies to engineer tendon/ligament-to-bone interface: Biomaterials, cells and growth factors☆

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ABSTRACT

Integration between tendon/ligament and bone occurs through a specialized tissue interface called enthesis. The complex and heterogeneous structure of the enthesis is essential to ensure smooth mechanical stress transfer between bone and soft tissues. Following injury, the interface is not regenerated, resulting in high rupture recurrence rates. Tissue engineering is a promising strategy for the regeneration of a functional enthesis. However, the complex structural and cellular composition of the native interface makes enthesis tissue engineering particularly challenging. Thus, it is likely that a combination of biomaterials and cells stimulated with appropriate biochemical and mechanical cues will be needed. The objective of this review is to describe the current *state-of-the-art*, challenges and future directions in the field of enthesis tissue engineering focusing on four key parameters: (1) scaffold and biomaterials, (2) cells, (3) growth factors and (4) mechanical stimuli.

Contents

1. Introduction

Interaction between soft and hard tissues is essential for musculoskeletal motion [1]. A specialized tissue interface, the enthesis, integrates tendon/ligament in bone and serves to facilitate joint motion [2]. The interface involves two materials of widely different mechanical properties: tendons/ligaments and bone. Tendons and ligaments are very strong in tension, while bone is optimized for compressive loading. Therefore, entheses are points of high stress concentration [3]. Consequently, entheses exhibit gradients in tissue organization, composition and mechanical properties that serve to (1) effectively transfer stress between mechanically dissimilar materials and (2) sustain the heterotypic cellular communications required for interface function and homeostasis [4]. Promoting the fixation of bone and soft tissue grafts with each other and at

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the implant site is particularly critical in the repair of injuries to ligaments and tendons. However, functional integration still remains a major challenge for orthopedic surgery.

A number of common orthopedic injuries require the repair of a ruptured tendon or ligament to its bony insertion. Two examples are the rotator cuff tendon in the shoulder and the cruciate ligaments in the knee, especially the anterior cruciate ligament (ACL). These critical junctions are not reestablished following surgical repair by autograft transplantation. In fact, the resulting neo-fibrovascular tissue is mechanically inferior. As a consequence, this procedure is associated with high recurrence rates [3,4]. This fibrous tissue exposes the insertion site to high mechanical stresses and increased failure risk, compromising graft stability and long term clinical outcome [1,5]. Consequently, there is a significant need to develop integrative graft fixation systems that can promote interface regeneration and facilitate functional graft-to-bone integration.

In the past decade, tissue engineering has emerged as a promising approach for musculoskeletal tissue repair and regeneration. Tremendous advances have been made whereby bone-, cartilage-, tendon- and ligament-like tissues have been engineered *in vitro* and *in vivo* using a combination of biomaterials, cells and/or growth factors [1]. More recently, the emphasis in the field of orthopedic tissue engineering has shifted from tissue formation to tissue function. The latter focuses on achieving biomimetic functionality of orthopedic grafts with the objective to move forward to clinical translation [6]. In the native enthesis, a strong mechanical attachment between tendon/ligament and bone is established *via* a structural gradient in the extracellular matrix (ECM). This structural gradient consists of a progressive change in collagen 3D fiber architecture and proteoglycan and mineral composition. In turn, these components are synthesized and maintained by a gradient of different cell types. This intricate multi-tissue organization makes interface tissue engineering particularly challenging. Therefore, a profound understanding of the structure–function relationship at the native enthesis tissue will be essential for the generation of biomimetic constructs. Accordingly, it is likely that a multiphasic scaffold system with multiple cell types as well as appropriate molecular and mechanical cues will be needed for the generation of a functional tendon/ligament-to-bone construct.

The objective of this review is to present current knowledge and strategies as well as limitations and future directions in the field of enthesis tissue engineering. The first part of the review focuses on the structure, function and development of the native enthesis tissue, especially emphasizing the relationship between ECM composition, structure and function. The second part deals with tissue engineering strategies and covers four main topics: (1) scaffold and biomaterials, (2) cells, (3) growth factors and (4) mechanical stimuli. Scaffold design and cell culture strategies follow the need to replicate the native enthesis structure, mechanical properties and cellular composition. Growth factor treatment and mechanical stimuli are mainly inspired by developmental cues and have the objective to direct cell differentiation and matrix deposition. Finally, current challenges and future directions in the field of interface tissue engineering will be discussed.

2. Enthesis: tissue interface at the tendon/ligament-to-bone attachment

2.1. Structure and function

From a histological point of view, tendon/ligament insertions can be characterized as fibrous or fibrocartilaginous. Fibrocartilaginous insertions are more common and include the bony attachments of the rotator cuff tendons, the ACL, and the Achilles tendon [2]. Because of their clinical relevance, this review will focus on fibrocartilaginous insertions.

Typically, four different zones can be distinguished within fibrocartilaginous entheses: tendon/ligament, non-mineralized fibrocartilage, mineralized fibrocartilage and bone (Figs. 1 and 2). Moving towards the insertion site, the first part of the enthesis

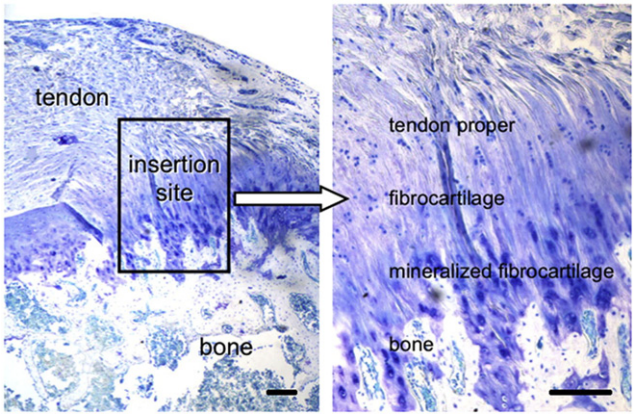

Fig. 1. Histological picture of the rat supraspinatus tendon-to-bone fibrocartilaginous enthesis. Toluidine blue-stained section from a rat supraspinatus tendon-to-bone insertion (scale bar = 200 mm). The four different zones of the fibrocartilaginous enthesis are indicated. Figure reprinted with permission from reference [192]. Copyright © 2011, Nature Publishing Group.

consists of fibrous connective tissue (tendon/ligament) characterized by the presence of parallel collagen fibers with interspaced elongated fibroblasts organized in arrays [3]. The ECM primarily consists of collagen type I and small amounts of proteoglycans [2]. The next zone is non-mineralized fibrocartilage, populated by round fibrochondrocytes arranged in rows. The ECM contains mostly collagen type II (typically characteristic of hyaline cartilage) as well as high levels of pericellular collagen type III, small amounts of collagen types I and X, and proteoglycans (mainly aggrecan) with associated chondroitin 4- and 6-sulfate glycosaminoglycans (GAGs) [3,5]. The third zone is mineralized fibrocartilage. This zone is populated by hypertrophic fibrochondrocytes, which are round and bigger than fibrochondrocytes. The ECM is composed mainly of collagen type II with significant amounts of collagen type X and aggrecan. Finally, the mineralized fibrocartilage merges into bone tissue containing osteoblasts, osteocytes and osteoclasts, together with collagen type I and high mineral content (69%) of which 99% is hydroxyapatite [3,5].

Although the insertion site has typically been defined as containing four zones, the different tissue regions are compositionally distinct, but structurally continuous. Consistently, Genin *et al.* [7] showed that the insertion site exhibits a gradual increase in mineral content with a corresponding gradual decrease in collagen fiber organization moving from the tendon/ligament to the bone. At the start of the tendon/ligament, collage fibers are aligned and parallel. These fibers start bending and intercrossing along the insertion, change their orientation and become more disorganized closer to the bone (Fig. 2) [3]. This competing gradation ensures a smooth mechanical stress distribution, improving the strength of the bonding and decreasing the risk of rupture or fracture [8]. In addition to a gradation in structural composition, there is also a change in cell type and morphology along the insertion. Enthesis fibrocartilage has been proposed to act as a barrier for cellular communication between tendon/ligament fibroblasts and osteocytes. This is due to the fact that fibrocartilage tissue is poorly vascularized and fibrochondrocytes do not express connexins and do not form gap junctions [9]. Thus, intercellular communication needs to take place indirectly *via* cell–matrix interactions or soluble factors. This is likely to contribute to the poor healing response observed at and near attachment sites [10].

There is abundant evidence to support that the structural and mechanical properties of the enthesis are a consequence of the mechanical stresses to which it is exposed. Of note, the similarity in cellular and ECM composition between the fibrocartilage and the hyaline cartilage indicates an adaptation to compression. Consistently, mechanical loading plays an important role during enthesis maturation and contributes to the generation of a structural and cellular gradient at the insertion