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Review

Regeneration and repair of tendon and ligament tissue using collagen fibre biomaterials

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ABSTRACT

Collagen fibres are ubiquitous macromolecular assemblies in nature, providing the structures that support tensile mechanical loads within the human body. Aligned type I collagen fibres are the primary structural motif for tendon and ligament, and therefore biomaterials based on these structures are considered promising candidates for mediating regeneration of these tissues. However, despite considerable investigation, there remains no collagen-fibre-based biomaterial that has undergone clinical evaluation for this application. Recent research in this area has significantly enhanced our understanding of these complex and challenging biomaterials, and is reinvigorating interest in the development of such structures to recapitulate mechanical function. In this review we describe the progress to date towards a ligament or tendon regeneration template based on collagen fibre scaffolds. We highlight reports of particular relevance to the development of the underlying biomaterials science in this area. In addition, the potential for tailoring and manipulating the interactions between collagen fibres and biological systems, as hybrid biomaterial–biological ensembles, is discussed in the context of developing novel tissue engineering strategies for tendon and ligament.

1. Introduction

Tendon and ligament regeneration has proved an elusive goal for tissue engineering owing to the specialised nature of these tissues and the high mechanical demands placed on the extracellular matrix (ECM) of these structures in the human body. There is a significant clinical need for augmentative and substitutional approaches that enhance the structural performance of damaged and degenerated tissues. The clinical problem may be chronic or acute, and may occur in a range of extra- and intra-articular environments. Furthermore, the mechanical demands supported by these structures vary within a wide range, making a generic reconstructive approach particularly challenging. Hence, several different surgical modalities and fixation strategies have been developed to partially replicate the structure and function of these critical tissues.

Surgical autografting procedures have been developed that can be used in a number of anatomical locations with considerable success; for example, the anterior cruciate ligament (ACL) can be replaced with bone–patellar–bone [1] or hamstring tissue [2]. However, these approaches inevitably result in damage and consequent morbidity at the donor site, necessitating a second invasive procedure [3,4]. Furthermore, sacrifice of these tissues may preclude a secondary revision operation and, perhaps most significantly, can cause pain and impair the native biomechanics of the harvest site [5]. Despite these drawbacks, autografts remain the "gold-standard" option for reconstructive tendon and ligament surgeries owing to the high mechanical strength of the tissues, excellent compatibility with the host tissues and their propensity to revascularise and remodel when transplanted [6]. However, the process of biological remodelling remains incompletely understood [7], with in vivo studies indicating a multifactorial nature of the repair, but clearly implicating mechanical loading [8] and hence the rehabilitation protocol as significant factors for the success of the procedure in humans [9].

Allograft tissues are increasingly being used clinically for the replacement of ruptured ACL, with some success reported for short-term outcomes [10]. However, these tissues are expensive, limited in availability and carry a limited risk of disease transmission from the donor. Furthermore, the sterilisation procedures employed to prepare these tissues are not standardised and can have a negative effect on biomechanical properties, leading to increased graft failure compared with autograft [11]. Xenograft

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materials have also been investigated for ACL reconstruction, with the overall aim being to provide full mechanical substitution. However, the ability to recapitulate the mechanical properties of the ACL was limited and rupture of the graft was observed in a number of cases [12]. Replacement of ligaments such as the ACL with synthetic polymers has been attempted and, whilst offering adequate initial mechanical strength, they have been largely unsuccessful owing to long-term deterioration of mechanical properties and inflammatory response due to release of degradation products [13–16].

Biological ECM materials have been investigated as degradable scaffolds for augmentative tendon repair, utilising decellularised xenograft or allograft tissues to promote native tendon repair processes [17]. There has been some in vivo evidence indicating that tendon remodelling can be mediated by these matrices [18]. However, despite their prevalence in the marketplace, ECM-derived materials have yet to find widespread acceptance for this application. The results from clinical usage of ECM materials have to date been mixed; the various commercially available products have been reviewed by Chen et al. who described the clinical issues with these materials in detail [19]. Significant drawbacks associated with the clinical use of xenograft/allograft ECM materials include poor mechanical properties compared to tendon (i.e. not mechanically supporting the repair), an antigenic effect of the residual non-collagenous ECM components, and potential for pathogenic transmission of virus or bacterial species [20]. In particular, the use of decellularised tissue can be associated with retention of antigenic components [21], which may cause an inflammatory response and potentially lead to serious clinical complications, such as post-operative oedema [22].

It is widely recognised that the natural three-dimensional matrix of connective tissues, which surrounds the musculoskeletal cells, plays a key biological and mechanical role [23]. Collagen is the principle load-bearing component of the extracellular matrix and confers high mechanical strength to tissues such as tendon. It is therefore theoretically capable of providing the structural properties required for load bearing in musculoskeletal tissues [24]. Unfortunately, the mechanical properties of collagen gels or porous collagen fabricated via either biological or physical means are vastly inferior to those of biological tissues. Furthermore, the ability of cells to rapidly synthesise load-bearing collagen matrix is limited [25]. Therefore, fabrication of aligned, mechanically strong collagen fibres constitutes an attractive first step in achieving a "bottom-up" fabrication method for biomaterials that mimic both the mechanical and biological ligament or tendon environment.

Aligned collagen fibre scaffolds based on self-assembled collagen were first reported by Kato et al. who found that a homogenised insoluble collagen gel could be extruded as a fibre by utilising fibrillogenesis in vitro to fabricate pseudo-native structures [26]. The alignment of fibrous constructs, on both the nano- and micro-scales, has been shown in numerous studies to direct the axial alignment of tissue synthesis and is a useful scaffolding motif for aligned tissues [27]. Furthermore, misalignment of collagen fibrils in repair tissue is believed to be associated with the mechanical inferiority of these tissues [28,29]. However, it has proved challenging to replicate the mechanical strength of native collagen in vitro and, importantly, to achieve adequate long-term mechanical properties in vivo, which has led to the exploration of synthetic [30] and collagen/synthetic composites [31]. It has remained an elusive goal for the field to replicate the structural features of the native collagen fibre, in particular the nanoscale features of the collagen fibril recently characterised in detail and summarised in Fig. 1B [37,110]. In this review we describe the recent research activity focusing on novel biomaterials fabrication processes using "bottom-up" aligned collagen scaffold fabrication. Particular emphasis is placed on recent developments in the field relating to the development of the collagen fibres and their underlying biomaterials science, crosslinking strategies and biological interactions. In addition, we discuss the interactions between collagen fibre materials and biological systems both in vivo and in vitro. Finally, we review the prospects for the development of collagen fibre/biologic combinations, which may enable novel modes of ligament and tendon regeneration and provide routes by which biofabricated scaffold materials can be developed.

2. Collagen fibre structures in tendon and ligament

Collagen is the most abundant protein in vertebrates, accounting for approximately 30% of all body proteins; it is a key and ubiquitous component of the ECM, providing the tensile strength required to fulfil the demanding biomechanical requirements of human tissues. Despite extensive investigations of synthetic materials for biomedical applications, purified and crosslinked collagen remains a preferred base material for ligament/tendon tissue engineering owing to its low antigenicity [32], chemotactic surface structure for fibroblasts [33], biocompatibility and proteolytic degradation pathways [34,35]. The crosslinking process may, however, reduce the number of cellular attachment sites (binding via cell surface receptors such as integrins) if the amino acid residues are utilised or occluded in the reaction; often making an inverse relation between mechanical properties and biocompatibility.

Tendons and ligaments connect bone to muscle and bone to bone, respectively, and are predominantly composed of type I collagen fibrils arranged such that they are axially load bearing. Their structural hierarchy, which is illustrated in Fig. 1A–C, is exceedingly complex [36]. Recent detailed fibre X-ray analysis of the collagen structure has characterised further complexity at the microfibrillar level, which indicates a super-twisted discontinuous right-handed helical structure that forms inter-unit packing via an interdigitated motif [37]. The fibril diameters vary from 10 to 500 nm, depending on a variety of biological factors. Tendon and ligament additionally exhibit a characteristic nanoscale axial banding pattern of light and dark regions, observed using transmission electron microscopy or atomic force microscopy with a 64–67 nm periodicity (Fig. 2C). This is due to the axial alignment of collagen fibrils that result from alternating overlap and gap zones, produced by the specific packing arrangement of the 300 nm long, 1.5 nm diameter collagen molecules. The ability of type I collagen to form striated fibrils is complex and involves specific charge–charge and hydrophobic interactions [38–40]. Microscopically, collagen is birefringent, and appears bright when viewed between crossed polarising filters due to its oriented structure. In addition, a further microscopic axial periodic zigzag pattern arises due to a crimped structure, in which straight fibrils are kinked with respect to the axis of orientation [41]. Under tensile stress, the crimps gradually disappear until they are no longer microscopically visible [28,42], suggesting that extension of tendon and ligament initially involves straightening of the crimps. As shown in Fig. 1D, this gives rise to a toe region in the force–time curve for human ligaments, which is followed by a linear region in which the tissue is reversibly extendable and then finally by yielding and failure.

Collagen type I can be extracted and purified from connective tissues and reconstituted. It is extracted as an insoluble crosslinked material or as soluble collagen, which can be either acid solubilised or enzyme solubilised (customarily pepsin extracted via cleavage of telopeptides). The extraction of collagen typically involves the acidification of a protein gel or suspension to form a fraction that can be reprecipitated. It has been known for over 50 years that collagen fibres assemble at physiological pH and temperature to form fibril bundles that exhibit the periodic nanoscale D-banding