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Review

Decellularized tendon as a prospective scaffold for tendon repair

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ABSTRACT

Tendon injuries impose significant clinical burdens on healthcare systems worldwide. At present, no therapeutic methods can cure tendon injuries in an ideal manner. With the development and improvement of decellularization technology, tendon extracellular matrix (ECM) can develop into novel scaffolds with potential for repairing injured tendons. Proper agents and decellularization protocols were developed to obtain tendon ECMs, and the method used to recellularize the tendon ECM was explored to create bio-functional neo-tendons for transplants. Further, preliminary testing was done to evaluate the reparative capacity of decellularized tendon scaffolds (DTSs). Here, we assess developments in tendon decellularization and recellularization processes, as well as the possibility for advancing DTSs into clinical applications based on recent findings.

Contents

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1. Introduction

Tendon laceration is a common type of soft tissue injury. It has been estimated that tendon injuries account for 30%–50% of sport-related injuries [1]. The annual expenditure on tendon injuries in the USA is estimated to be 30×10^9 dollars, while European healthcare expenditures on tendon injuries exceed 115×10^9 euros per year [2]. Because of the lack of blood supply and poor intrinsic repair abilities of tenocytes, tendon injuries undergo slow regeneration [3]. The natural repair processes in tendons can be accelerated by fibroblasts and blood vessels, which usually cause the formation of granulated tissue and inevitably lead to scar formation. As a result, tendon fibers become disordered when injured, causing a decrease in the mechanical properties of tendons and further impacts on their motor functions [4]. In addition, the invasion of peripheral tissues may lead to tendon adhesion [5]. Scarring and adhesion are two problems that remain to be solved. Relatively good effects have been achieved by direct suturing [6], growth factor injection [7], and tendon cell injection [8] when treating mild tears, while tendon tissue engineering is a better choice for severe tendon lacerations [9]. After over ten years of development of tendon tissue engineering, many artificial tendon materials have been derived from polylactic acid [10], silk [11], and collagen [12]. Furthermore, some new technologies were also invented, such as electrospinning [13], which enables the arrangement of material fibers much more closely to that found in the human body.

Despite rapid developments with artificial bionic scaffolds in recent years, no synthetic material has been developed that can replace natural tendon, owing to the complex composition of the extracellular matrix (ECM) and its particular biomechanical characteristics. Thus, a new strategy has been developing using intact ECM as the scaffold, rather than mimicking it. A technique known as decellularization was invented for this purpose, which uses detergents, enzymes, and physical methods to remove cells from the ECM [14]. Decellularized ECM material has shown great potential and special advantages over other biomaterials. Transplanted decellularized heart valves [15], kidneys [16], and livers [17] have shown good biological functions.

Decellularized materials (such as decellularized dermis [18,19] and small intestinal submucosa [20]) have been used to repair severe tendon defects in clinical settings. Decellularized tendon scaffold (DTS) materials, which usually contain native tendon ECM with the cells removed, have been studied previously and are expected to facilitate better repair results. The retained tendon ECM shows striking similarity to the native tendon, in terms of its bioactive components, collagen arrangement, and biomechanical characteristics [21]. Furthermore, DTSs retain many active growth factors [22] and have good biocompatibility, which promotes cell growth and differentiation [23]. In recent years, various studies with DTS have shown its special advantages in tendon tissue engineering. This review summarizes recent developments with DTSs and discusses possibilities for DTS applications in clinical tendon regeneration.

2. Overview of tendon decellularization protocols

Decellularization protocols are applied to native tendons to remove the constituent cells and obtain tendon ECM. Ideally, all cell components should be removed while preserving the ingredients, structural and mechanical properties of the tendon ECM to the maximum extent. In addition, toxic agents used in decellularization protocols should be removed to ensure the biocompatibility of DTSs.

2.1. SDS and TnBP

After several years of research, some chemical agents such as sodium dodecyl sulfate (SDS) and tri (n-butyl) phosphate (TnBP) have been found suitable for tendon decellularization. TnBP and SDS both have the capacity to disrupt protein-protein interactions and, thus, can facilitate the removal of cells [14,24]. Some researchers prefer TnBP (1% concentration) over SDS (0.5% concentration) because of its minimal disruption of the ultrastructure. However, the extensibility of DTSs is increased after TnBP treatment. In addition, DNA content assays have revealed that TnBP is ineffective at removing DNA from tissues [25] In contrast, SDS is more effective than TnBP both in eliminating cells and DNA from tissues. The effective SDS concentration can be decreased to 0.03% [26], while 0.1% SDS was found optimal for tendon decellularization [25]. The structure disruption caused by SDS may occur because many researches use an improperly high SDS concentration, with 0.5% SDS used as the lowest concentration during decellularization [24,27]. In fact, 0.1% SDS is equally effective compared with 1% SDS in removing cells [25]. Increasing SDS concentrations only cause destruction of the ECM. The apparent effect of SDS on the tendon ECM is a pronounced opening of spaces between the aligned collagen fibers [25,27]. However, this effect may be beneficial, as one study provided evidence that the "opening effect" contributes to ECM recellularization [28] (discussed further below).

2.2. Enzymes

As chemical detergents have little selectivity and are likely to alter or damage the ECM, enzymes provide high specificity in removing cell residues and undesirable ECM constituents. Therefore, enzymes can potentially produce better DTSs. Common enzymes used for tendon recellularization include nucleases, trypsin, collagenase A, and proteases. Nucleases, especially DNase I, are used to eliminate diffuse nuclear fragments following detergent treatment, with the aim of decreasing the DNA content in the scaffold [28–30]. The disadvantage of using nucleases is the difficulty in removing the residual DNA from large tissue samples, especially in dense tissues like tendons [31]. A good strategy for addressing this issue is to cut the tendon into slices [22,32]. Trypsin cleaves peptide bonds on the carboxy-terminal side of Arg and Lys residues [33,34], but cannot cleave intact triple helical collagen [35], which makes it a potential agent for tendon decellularization. Promising results have been obtained after exposing tendon tissue to trypsin as an initial step because it disrupts the ECM surrounding the collagen fiber, creates tiny channels, and facilitates the subsequent infiltration of decellularization agents into the deep regions of tendons and the separation of cells from the ECM [21,36,37]. Previous research showed that after treatment with trypsin, collagenase A, and protease, cells could be easily removed by ultrasonic cleaning alone [38].

2.3. Peracetic acid and snap freezing-thawing

Although the chemical agents and enzymes mentioned above have been relatively effective, some additional problem need to be overcome. Because tendon is a dense tissue without an abundant pipe network such as vessels and bronchia, chemical agents and enzymes have to infiltrate into the tissue by passive diffusion. As a result, the tendon surface is exposed to higher chemical concentrations and a longer processing time than the core. The inhomogeneous effects of the agents lead to surface damage and cells remaining in the core, and the former