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Multilayered polycaprolactone/gelatin fiber-hydrogel composite for tendon tissue engineering ☆

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ABSTRACT

Regeneration of injured tendon and ligament (T&L) remains a clinical challenge due to their poor intrinsic healing capacity. Tissue engineering provides a promising alternative treatment approach to facilitate T&L healing and regeneration. Successful tendon tissue engineering requires the use of three-dimensional (3D) biomimetic scaffolds that possess the physical and biochemical features of native tendon tissue. We report here the development and characterization of a novel composite scaffold fabricated by co-electrospinning of poly-ε-caprolactone (PCL) and methacrylated gelatin (mGLT). We found that photocrosslinking retained mGLT, resulted in a uniform distribution of mGLT throughout the depth of scaffold and also preserved scaffold mechanical strength. Moreover, photocrosslinking was able to integrate stacked scaffold sheets to form multilayered constructs that mimic the structure of native tendon tissues. Importantly, cells impregnated into the constructs remained responsive to topographical cues and exogenous tenogenic factors, such as TGF-β3. The excellent biocompatibility and highly integrated structure of the scaffold developed in this study will allow the creation of a more advanced tendon graft that possesses the architecture and cell phenotype of native tendon tissues.

Statement of Significance

The clinical challenges in tendon repair have spurred the development of tendon tissue engineering approaches to create functional tissue replacements. In this study, we have developed a novel composite scaffold as a tendon graft consisting of aligned poly-ε-caprolactone (PCL) microfibers and methacrylated gelatin (mGLT). Cell seeding and photocrosslinking between scaffold layers can be performed simultaneously to create cell impregnated multilayered constructs. This cell-scaffold construct combines the advantages of PCL nanofibrous scaffolds and photocrosslinked gelatin hydrogels to mimic the structure, mechanical anisotropy, and cell phenotype of native tendon tissue. The scaffold engineered here as a building block for multilayer constructs should have applications beyond tendon tissue engineering in the fabrication of tissue grafts that consist of both fibrous and hydrogel components.

1. Introduction

Tendons and ligaments are prone to injuries such as rupture and laceration due to their load-bearing nature [1,2]. In cases of severe tendon injury, surgical intervention is employed to repair or replace the damaged tendon with autografts, allografts, xenografts, or prosthetic devices [3–5], for the natural healing process is slow and insufficient [6,7]. To date, the clinical outcomes of tendon repair remain limited and unsatisfactory due to donor site morbidity, high failure rates, risk of injury recurrence, and limited long-term

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function restoration [8–10]. These limitations have spurred the development of tendon tissue engineering approaches, which apply combination of cells, scaffolds and bioactive molecules, as a promising strategy to create functional tissue replacements or to enhance the innate healing process [11,12]. Ultimately, tendon tissue engineering aims at improving the quality of healing in order to fully restore tendon structure and function [13].

Tendon tissues are composed of densely packed aligned collagen fibrils that connect muscle to bone [7,14]. Therefore, aligned nano- and micro-fibrous scaffolds fabricated by electrospinning have been extensively explored in attempts to recapitulate the mechanical and topographical characteristics of native tendon tissue [15–17]. Electrospun poly-ε-caprolactone (PCL) scaffolds are frequently used in tendon tissue engineering as well as applications for other soft tissues. PCL is an aliphatic linear polyester approved by the U.S. Food and Drug Administration for clinical use [18]. It is biocompatible, bioresorbable and a low-cost synthetic polymer. Of equal importance, PCL exhibits low degradation rate due to its semi-crystalline and hydrophobic nature [19,20], making it a suitable graft material to facilitate the relatively slow healing process of injured tendons [21,22]. However, the hydrophobic nature of PCL often results in poor wettability [23], inadequate cell attachment, and poor tissue integration [24] when used in tissue engineering. Moreover, as a synthetic polyester, its lack of bioactivity is a major challenge for PCL to direct cell behavior after seeding due to the absence of cell-binding motifs found in natural extracellular matrix (ECM) proteins [25].

Hydrogels prepared from collagen and its derivative, gelatin, represent another class of scaffolds for regenerating and repairing a wide variety of tissues and organs [26,27]. Unlike other types of scaffolds, hydrogels retain a large volume of water and thus provide a highly hydrated environment similar to that in native tissues. Cells encapsulated within collagen/gelatin hydrogels can be easily distributed homogeneously by simple mixing during gel preparation [28,29]. Importantly, collagen and gelatin, as constituents of natural ECM, better mimic at least in part the native tissue microenvironment, as compared to synthetic polymers [30,31]. Nevertheless, improvement in the mechanical properties and introduction of topographical cues are needed to apply these hydrogels to tendon grafts that aim at reproducing the mechanical and structural features of native tendon tissues.

The organization of native ECM may be viewed as a cell-containing hydrogel reinforced by structural fibers. An engineered scaffold consisting of hydrogels and electrospun fibers may thus be considered as a biomimetic of the ECM. For example, a microfiber-reinforced silk hydrogel displayed a greatly improved modulus compared to a fiber-free hydrogel [32]. In addition, hydrogels composed of natural proteins could provide the bioactive motifs absent from synthetic polymeric scaffolds to enhance control of cell binding and fate determination [28,33]. In terms of tendon tissue engineering, an ideal composite scaffold consisting of hydrogel and fibrous scaffold has yet to be developed. Consequently, little is known about the effects such a composite scaffold may have on the activities of encapsulated cells.

In this study, we have developed a novel composite scaffold as a tendon graft consisting of electrospun PCL microfibers and methacrylated gelatin (mGLT). We have optimized the retention of mGLT by photocrosslinking and its integration with the fibrous scaffold. Simultaneous cell seeding and photocrosslinking between scaffold layers were performed to create cell-impregnated multilayered constructs, and their mechanical properties and architecture and the activity of encapsulated cells were assessed. Our results show that this novel cell-scaffold construct combines the advantages of PCL nanofibrous scaffolds and gelatin hydrogels to mimic the mechanical feature, structure and cell phenotype of native tendon tissue.

2. Materials and methods

2.1. Synthesis of methacrylated gelatin

Methacrylated gelatin (mGLT) was synthesized using an established protocol with slight modification [29,34]. Gelatin (GLT, Sigma–Aldrich) was dissolved in deionized H_2O at 37 °C (30%, w/v). Methacrylic anhydride (Sigma–Aldrich) was then added dropwise into the mixture at 37 °C under mild agitation to react with amine groups on GLT for 24 h (Supplementary Fig. S1 A). Reacted mGLT solution was dialyzed against water to completely remove low molecular-weight byproducts using 3500 NMWCO dialysis cassettes (Thermo Scientific). Dialyzed mGLT was lyophilized and stored desiccated for future use. The methacrylation rate of the product was ~80% [34]. The visible light (VL)-activated photo-initiator lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was synthesized as described by Fairbanks et al. [35].

2.2. Fabrication of composite scaffolds

Composite scaffolds containing interspersed PCL and mGLT fibers were produced using dual electrospinning [36]. PCL particles (70–90 k, Sigma–Aldrich) were dissolved in 2,2,2-trifluoroethanol (TFE, Sigma–Aldrich, 18% w/v). Dehydrated mGLT was dissolved in 95% TFE (20% w/v). Each polymer solution was extruded through a 22-gauge blunt-tip needle at 2 mL/h for 1 h. The spinnerets were charged with an optimized DC potential (8 kV for PCL and 15 kV for mGLT, respectively) and aligned in opposing positions on each side of the collection mandrel with a distance of 15 cm between the needle tip and mandrel (Fig. 1). The scaffold was stored desiccated under vacuum to remove residual solvent. To retain mGLT in the composite scaffold, 0.5% LAP solution was cast onto dry composite scaffold surface at 15 μl/cm^2 and allowed to spread until the scaffold was completely wet. LAP was then activated by exposure to VL irradiation (450–490 nm) to photocrosslink the methacrylate groups of the dissolved gelatin in the scaffold (Fig. 1, Supplementary Fig. S1 B).

2.3. Imaging of composite scaffolds

Polymers were fluorescently labeled to track the presence and interspersion of the two distinct fiber populations: PCL solution was mixed with 0.1% (v/v) Vybrant® DiI Cell-Labeling Solution (DiI, Life Technologies), and non-methacrylated GLT was conjugated with fluorescein 5(6)-isothiocyanate (FITC, Sigma–Aldrich) before dissolution, respectively. Fibers were dual-electrospun onto glass slides for 5 min and imaged before and after wetting using an Olympus CKX41 inverted fluorescent microscope equipped with a CCD camera. Additionally, scaffold surface was examined by scanning electron microscopy (SEM, field emission, JEOL JSM6335F) operated at 3 kV accelerating voltage and 8 mm working distance.

2.4. Histological examination of composite scaffolds

Picrosirius red staining was employed to assess gelatin retention and distribution within scaffolds. Composite scaffolds before and after photocrosslinking were washed in PBS at 37 °C overnight under mild agitation, frozen-embedded in OCT compound (4583 Scigen Scientific), and cryosectioned at 15 μm thickness using a Leica CM 1850 cryotome. Sections were washed in PBS and stained with 0.1% sirius red in saturated picric acid (Electron Microscopy Sciences) for 1 h. To visualize impregnated cells, cryosections of fixed, cell-seeded scaffolds were incubated with ethidium homodimer-1 (EthD-1, Life Technologies) to label cells via DNA binding.

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