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Alignment of collagen fiber in knitted silk scaffold for functional massive rotator cuff repair

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ABSTRACT

Rotator cuff tear is one of the most common types of shoulder injuries, often resulting in pain and physical debilitation. Allogeneic tendon-derived decellularized matrices do not have appropriate pore size and porosity to facilitate cell infiltration, while commercially-available synthetic scaffolds are often inadequate at inducing tenogenic differentiation. The aim of this study is to develop an advanced 3D aligned collagen/silk scaffold (ACS) and investigate its efficacy in a rabbit massive rotator cuff tear model. ACS has similar 3D alignment of collagen fibers as natural tendon with superior mechanical characteristics. Based on ectopic transplantation studies, the optimal collagen concentration (10 mg/ml), pore diameter (108.43 ± 7.25 μm) and porosity (97.94 ± 0.08%) required for sustaining a stable macro-structure conducive for cellular infiltration was determined. Within in vitro culture, tendon stem/progenitor cells (TSPCs) displayed spindle-shaped morphology, and were well-aligned on ACS as early as 24 h. TSPCs formed intercellular contacts and deposited extracellular matrix after 7 days. With the in vivo rotator cuff repair model, the regenerative tendon of the ACS group displayed more conspicuous native microstructures with larger diameter collagen fibrils (48.72 ± 3.75 vs. 44.26 ± 5.03 nm) that had better alignment and mechanical properties (139.85 ± 49.36 vs. 99.09 ± 33.98 N) at 12 weeks post-implantation. In conclusion, these findings demonstrate the positive efficacy of the macroporous 3D aligned scaffold in facilitating rotator cuff tendon regeneration, and its practical applications for rotator cuff tendon tissue engineering.

Statement of Significance

Massive rotator cuff tear is one of the most common shoulder injuries, and poses a formidable clinical challenge to the orthopedic surgeon. Tissue engineering of tendon can potentially overcome the problem. However, more efficacious scaffolds with good biocompatibility, appropriate pore size, favorable inductivity and sufficient mechanical strength for repairing massive rotator cuff tendon injuries need to be developed. In this study, we developed a novel macroporous 3D aligned collagen/silk scaffold, and demonstrated that this novel scaffold enhanced the efficacy of rotator cuff tendon regeneration by inducing aligned supracellular structures similar to natural tendon, which in turn enhanced

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cellular infiltration and tenogenic differentiation of stem/progenitor cells from both the tendon itself and surrounding tissues. Hence, it can potentially be a clinically useful application for tendon tissue engineering.

1. Introduction

Rotator cuff tendon injury is one of the most common types of injuries encountered during overhead activities and sports, often resulting in much pain and physical debilitation [1,2]. About 10% to 40% of all rotator cuff tears are massive rotator cuff tears that present a formidable clinical challenge to the orthopedic surgeon, and which often require implantable devices for therapy [3]. Tissue grafts, particularly allograft is the current gold standard in clinical practice. However allogeneic tendon-derived decellularized matrices do not have appropriate pore size and porosity to facilitate cell infiltration [4]. Commercial products based on the decellularized matrix of other tissues, such as Restore®, Bio-Blanket® and TissueMend®, are also used in rotator cuff repair [5]. However, their effects on tenogenic differentiation are usually weaker than tendon-derived decellularized matrices [6]. In addition, synthetic scaffolds have the drawback of poor cell adhesion, and can induce chronic inflammation [7,8]. Hence, more efficacious scaffolds with good biocompatibility, appropriate pore size, favorable inductivity and sufficient mechanical strength for facilitating repair of massive rotator cuff tendon injuries need to be developed.

Tissue engineering of tendon can potentially overcome some of these deficiencies and promote the healing of tendon injury [9,10]. One of the key components of tendon tissue engineering is the scaffold. Collagen has many advantageous properties such as ubiquity, low antigenicity, biocompatibility and biodegradability, and has been widely utilized as a scaffold biomaterial [11]. In particular, type I collagen is the major component of tendon extracellular matrix, and has been extensively utilized in tendon tissue engineering [12]. Nevertheless, regenerated tendon tissue within sponge collagen scaffold is disordered and irregular, which can have a negative impact on the biomechanical function of the regenerated tendon [13]. Hence, it is imperative to further improve the collagen scaffold utilized for tendon repair.

Recent studies showed that aligned scaffolds can exert a profound influence on cell morphology and tendon-related gene expression, resulting in enhancement of tenogenic differentiation both in vitro and in vivo with animal injury models [14–17]. However, aligned scaffolds fabricated by the electrospinning procedure are nanoporous, usually having low porosity with small pores between fibers. These pores are smaller than the typical cell size. As a result, cells can only grow on the top of the scaffold, leading to low cell infiltration rates being observed in vivo [18]. Although much effort has been devoted to increasing the porosity and pore size [19,20], a robust and implantable aligned scaffold has not yet been developed, due mainly to the reduction of mechanical strength with increasing pore size and porosity.

Because pure collagen scaffold exhibit poor mechanical properties in vivo, it is necessary to combine it with a material that possesses good mechanical strength [13]. Silk is a FDA -approved material, which has been widely utilized in tissue engineering due to its good biocompatibility and mechanical strength [21]. In particular, knitted silk scaffold can meet many of the requirements for tendon tissue engineering [22].

The aim of this study is to fabricate a macroporous 3D aligned collagen-silk scaffold with optimal pore size (several hundred micrometers), adequate porosity (higher than 90%) [20], favorable inductivity (enhancing tenogenic differentiation) and sufficient mechanical strength (no tear after implantation in vivo) [13], and to investigate its effect on tendon remodeling during functional shoulder repair in a rabbit massive rotator cuff defect model.

2. Materials and methods

2.1. Scaffold fabrication

Raw Bombyx mori silk fibers were purchased from Zhejiang Cathaya International Co. Ltd. The knitted silk was fabricated as described previously [13]. Briefly, the knitted scaffold was fabricated using 12 yarns (1 filament/yarn) of silk fibers on a knitting machine. Plain knitted silk scaffolds were fabricated with 21 stitches per centimeter. The knitted scaffolds were then processed to extract sericin, by rinsing three times in an aqueous solution containing 0.02 M Na_2CO_3 at 90 °C and 100 °C for 60 min. The pore size was approximately 1 × 1 mm.

Insoluble type I collagen was derived from pig Achilles' tendon using neutral salt and dilute acid extractions with enzyme treatment [13,23]. Type III collagen was removed by centrifugation after salt fractionation, and type I collagen was drawn off the lower portion. The macroporous 3D aligned collagen scaffold was produced by unidirectional freezing technology in a horizontal direction. The collagen was held in perspex molds (20 × 20 × 5 mm; type I, pH 3.2, 6/10/18 mg/ml) with L shaped aluminum rods (including two heads: long head and short head). Freezing temperature was created by liquid nitrogen (LN2) through the short head of the aluminum rods, and transmitted along the aluminum rod. A temperature gradient was created, and the collagen froze from the long head of the aluminum rod, and became aligned. To produce collagen sponge scaffolds, the collagen was held in perspex molds (20 × 20 × 5 mm; type I, pH 3.2, 6/10/18 mg/ml) without aluminum rods, and frozen at −80 °C for 12 h. To fabricate macroporous 3D aligned collagen/silk scaffold, the collagen was initially held in perspex molds (20 × 20 × 2.5 mm; type I, pH 3.2, 6/10/18 mg/ml). Then, the knitted silk was placed flat on the top of the collagen solution. Finally, the rest of the collagen (20 × 20 × 2.5 mm; type I, pH 3.2, 6/10/18 mg/ml) was added to the knitted silk before freezing. The scaffolds were freeze-dried under vacuum (Heto PowerDry LL1500) for 48 h to remove water, and were then crosslinked by dehydrothermal treatment (22 °C for 24 h, 110°Cfor 72 h, and 65°Cfor 24 h, at vacuum pressure <10 mbar) [13]. The structural morphology of the scaffolds (longitudinal section) was characterized by light microscopy and scanning electron microscopy (SEM), and the biomechanical properties of the scaffolds were evaluated by an Instron tension/-compression system.

The porosity of the scaffold was calculated according to the following formula (n = 5 for each group):

$$\text{Porosity } (\%) = (1 - V_c/V_t) \times 100\% = (1 - m/\rho) \times 100\%$$

where V_c is the volume occupied by the collagen, V_t is the total volume of the scaffold, m is the mass of the scaffold, ρ is the density of anhydrous collagen, assumed to be 1.3 g/ml [24].

The pore diameter of scaffold was measured by image analysis software (Image-Pro Plus, Rockville, MD, USA).