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Controlled release of collagen-binding SDF-1α from the collagen scaffold promoted tendon regeneration in a rat Achilles tendon defect model

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ABSTRACT

It had been demonstrated that stromal cell-derived factor-1α (SDF-1α) could promote *in situ* tendon regeneration by recruiting endogenous cells. However, native SDF-1α diffuses too fast *in vivo*, reducing its local concentration and efficacy. In this study, we prepared a recombinant SDF-1α containing a collagen-binding domain (CBD-SDF-1α) and developed a functional collagen scaffold by tethering CBD-SDF-1α on the collagen scaffold for *in situ* tendon regeneration. CBD-SDF-1α could induce the migration of mesenchymal stem cells, dermal fibroblasts and Achilles tendon fibroblasts *in vitro*, and achieve controlled release from the collagen scaffold. In a rat Achilles tendon defect model, the functional scaffold could increase the recruitment of CXCR4 positive fibroblast-like cells and the deposition of Tenascin-C at 7 days after implantation. After 4 and 12 weeks, the functional collagen scaffold could promote the expression of type I collagen, increase the diameters of collagen fibrils and improve the mechanical properties of regenerated tendons. Hence, the functional scaffold increased the efficacy of tendon regeneration by controlling release of SDF-1α, enhancing the recruitment of fibroblast-like cells and providing instructive microenvironment and mechanical support for tendon regeneration. Therefore, CBD-SDF-1α-modified collagen scaffold could serve as a practical application for tendon regeneration.

1. Introduction

Tendons are soft tissues that connect muscles to bones. Due to various physical exercises, tendon diseases and accidents, tendon injury has a high morbidity [1,2]. At present, there is no effective treatment for complete Achilles tendon rupture because the limited regenerative capacity of tendon and the distal retraction of rupture results in the formation of a gap [3–5]. Current treatment for tendon injuries usually have some side effects, such as autograft often results in secondary injury and postoperative complications, while allograft, xenografts and ligament prostheses have the risk of immune rejection, disease transmission, poor graft integration and scar formation [6–9]. The formation of fibrotic scar tissue cannot satisfy the mechanical properties of tendon, often leading to secondary rupture [10]. Therefore, the ideal treatment for tendon injury is to promote tendon regeneration.

With the development of regenerative medicine, biomaterials and stem cells have been widely used in the study of tendon regeneration. The biomaterials can connect two ends of the broken tendon, provide microenvironment and mechanical support for tendon regeneration [11–13]. Biomaterials used for tendon regeneration should have good biocompatibility and mechanical properties, and promote cell adhesion and growth. Although many studies have shown that scaffolds can promote tendon

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regeneration to a certain extent, the structure and function of regenerative tendons are far inferior to that of natural tendons [14,15], implying that scaffolds alone without biological activity cannot effectively promote tendon regeneration. Some studies have shown that biomaterials combined with mesenchymal stem cells (MSCs), dermal fibroblasts (DFs) or Achilles tendon fibroblasts (ATFs) can be more effective in promoting tendon regeneration [16–19]. However, the application of exogenous cell transplantation is restricted by cell source, immune rejection and ethic, and often leads to ectopic osteogenesis, this is not conducive to clinical application and transformation [20]. In addition, the survival and differentiation of exogenous cells is largely limited by the injured microenvironment.

Recently, Minami et al. found that the expression of SDF-1α increased after tendon injury, which could promote the *in situ* tendon regeneration by recruiting endogenous MSCs, DFs and ATFs to the injured site [21]. SDF-1α is one kind of chemokines, its receptor CXCR4 is expressed on many kinds of stem cell surfaces , such as MSCs, hematopoietic stem cells (CSCs), cardiac stem cells (CSCs) and fibroblasts and so on. Many studies have shown that localized delivery of SDF-1α at the injured site can promote the regeneration of multiple tissues by recruiting endogenous stem cells, including heart, nerve, bone and tendon, which can overcome many of shortcomings in the clinical application of exogenous stem cell transplantation [22–24]. Imitola*et al.* reported that SDF-1α/CXCR4 axis played a vital role in the migration of neural stem cells to injured area in central nervous system [25], which also exerted a major role in vascularization after myocardial infarction [26].

Collagen is the main component of tendon. Collagen scaffold has good biocompatibility and mechanical properties, which is an ideal material for tendon regeneration. Previous studies have reported that collagen fibers prepared by electrospinning can promote tendon repair [27–29]. Hence, collagen scaffold loads with SDF-1α may promote *in situ* tendon regeneration by recruiting endogenous MSCs, ATs and so on. Shen et al. reported that collagen gel incorporated with SDF-1α could promote tendon regeneration in a rat Achilles tendon defect model [30]. However, the mechanical strength of collagen fiber or gel is too weak to meet the mechanical properties of tendon. Moreover, native SDF-1α (NAT- SDF-1α) diffuses and degrades too rapidly *in vivo*, reducing its local concentration and efficacy, and causes some side effects. In order to solve this problem, chemical cross-linking was used to retain SDF-1α on the scaffold, but which would affect its bioactivity and the structure of scaffold.

In the present study, we developed a recombinant SDF-1α containing a collagen-binding domain (CBD), named CBD-SDF-1α, which could specifically bind to collagen and achieve controlled release. CBD is a heptapeptides (TKKTLRT) derived from the collagen-binding domain of collagenase, which has special binding ability to collagen and achieves sustained release *in vitro* and *in vivo* [31]. Subsequently, a functional collagen scaffold was fabricated by tethering CBD-SDF-1α on the collagen scaffold for tendon injury repair. The controlled release of CBD-SDF-1α from the collagen scaffold could increase the local concentration of SDF-1α at the injured site and prolong the role of time, which may enhance the recruitment of endogenous stem cells and fibroblasts. Moreover, the collagen scaffold could provide a 3-dimensional microenvironment for cells adhesion, and provide a mechanical support to inhibit the retraction of the broken tendons. In this study, collagen derived from the bovine tendons consisting mainly of type I and III collagen was used to create the scaffold, which might facilitate the adhesion, survival and differentiation of cells that promote tendon regeneration. Our results showed that CBD-SDF-1α could induce the migration of MSCs, DFs and ATFs, and achieve controlled release from collagen scaffold *in vitro*. In a rat Achilles tendon defect model, CBD-SDF-1α-modified collagen scaffold could enhance the recruitment of endogenous CXCR4 positive ($CXCR4^+$) fibroblast-like cells and the deposition of Tenascin-C. Importantly, the functional scaffold could promote the expression of type I collagen and improve the mechanical properties of regenerated tendons. This method of promoting endogenous regeneration of the tendons could avoid the drawbacks in the clinical application of exogenous cell transplantation, and it was more beneficial to clinical transformation and application.

2. Materials and methods

2.1. Preparation of CBD-SDF-1α

CBD-SDF-1α was prepared according to our previous study [32]. Briefly, CBD was incorporated into the C-terminus of NAT-SDF-1α and separated by a linker sequence to avoid affecting the bioactivity of SDF-1α (Fig. 1A). Subsequently, CBD-SDF-1α gene was inserted into the pET28a vector and expressed in BL21 (DE3) Escherichia coli. The protein was purified using Akta system and nickel column affinity chromatography.

2.2. Cell culture

Rat bone marrow MSCs, DFs and ATFs were isolated from male rats weighing from 100 ~ 120 g. MSCs were cultured and expanded with DMEM media plus 10% fetal bovine serum. DFs and ATFs were cultured and expanded with RPMI1640 media plus 10% fetal bovine serum. All media was replaced every two days and the third to fifth passages were used for subsequent cell migration assay.

2.3. Migration assay

The transwell system was used to evaluate the migration capacity of MSCs, DFs and ATFs mediated by CBD-SDF-1α, as previously described [32]. Briefly, 1×10^5 cells in 200 μL basal media were added into the upper chamber. To induce chemotaxis, 600 μL basal media containing CBD-SDF-1α or NAT-SDF-1α (150 ng/mL) was added to the lower chamber. After incubation at 37 °C for 12 h, the migrated cells were fixed with 4% paraformaldehyde and stained with crystal violet. The migration rate was calculated using the following formula: Migration rate = Number of migrated cells in CBD-SDF-1α or NAT-SDF-1α group/that of in control group.

2.4. Preparation and characterization of collagen scaffold

Collagen derived from bovine tendons was used to create the scaffolds, consisting mainly of type I and III collagen. Collagen scaffolds were prepared as previously described [33]. Briefly, collagen was dissolved in 0.5 M acetic acid for 8 h at 4 °C, and homogenized in a blender for 15 min, followed by neutralization with 4 M NaOH. The homogeneous solution was dialyzed against deionized water for 5 days to remove any residual salts and then lyophilized. The morphology of collagen scaffold was characterized by scanning electron microscopy (SEM). The porosity and pore size of the collagen scaffold were tested using a Mercury Injection Apparatus (AutoPore V9620, Micromeritics, America), and the mechanical properties were tested using a tension system (HY-1080, Hengyi, China) at a tensile speed of 2 mm/min. The swelling ration (SR) of the collagen scaffold was tested according to the previously described [34]. Briefly, collagen scaffolds were immersed in PBS (pH 7.4) at room temperature for 12 h to reach swelling equilibrium. Swollen samples were then separated from unabsorbed water by filtering through a 100-mesh screen under