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Fiber-based tissue-engineered scaffold for ligament replacement: design considerations and in vitro evaluation

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Abstract

The anterior cruciate ligament (ACL) is the major intraarticular ligamentous structure of the knee, which functions as a joint stabilizer. It is the most commonly injured ligament of the knee, with over 150,000 ACL surgeries performed annually in the United States. Due to limitations associated with current grafts for ACL reconstruction, there is a significant demand for alternative graft systems. We report here the development of a biodegradable, tissue-engineered ACL graft. Several design parameters including construct architecture, porosity, degradability, and cell source were examined. This graft system is based on polymeric fibers of polylactide-*co*-glycolide 10:90, and it was fabricated using a novel, three-dimensional braiding technology. The resultant microporous scaffold exhibited optimal pore diameters (175–233 μm) for ligament tissue ingrowth, and initial mechanical properties of the construct approximate those of the native ligament.

Keywords: Porosity; Degradable; Polymer; Anterior cruciate ligament; Ligament repair; Tissue engineering; Ligament and ligament tissue engineering

1. Introduction

The anterior cruciate ligament (ACL) is a commonly injured ligament of the knee, with over 250,000 patients each year diagnosed with a torn ACL, and approximately 150,000 ACL surgeries performed annually [1,2]. The ACL is an intraarticular ligament that controls normal motion and acts as a joint stabilizer. It connects the femur to the tibia and is completely enveloped by synovium. Due to the ACL's intrinsically poor healing potential and limited vascularization, ACL ruptures do not heal and surgical intervention is usually required. Current treatment modalities utilizing autogenous grafts such as bone–patellar tendon–bone and hamstring tendon have demonstrated clinically functional outcomes [3–9]. However, autogenous grafts are limited by donor site-related problems such as harvest site infection, nerve injury, and patellar fracture. Allografts are restricted in use due to the potential for infectious disease transfer and unreliable graft incorporation [10]. There are several commercially available synthetic ACL grafts, including the Gore Tex prosthesis, the Stryker–Dacron ligament, and the Kennedy ligament augmentation device (LAD) [5,11–13]. Although these synthetic grafts exhibit excellent short-term results, the long-term clinical outcome is poor due to mechanical mismatch, poor abrasion resistance, high incidence of fatigue failures, and limited integration between the graft and host tissue [14–17]. Clearly, alternative ACL replacement and reconstruction methods would be advantageous.

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There is a growing interest in tissue-engineered solutions to musculoskeletal injuries. Tissue engineering may be defined as the application of biological, chemical, and engineering principles toward the repair, restoration, or regeneration of living tissues using biomaterials, cells, and factors alone or in combination [18]. The ideal ACL replacement scaffold should be biodegradable, porous, biocompatible, exhibit sufficient mechanical strength, and able to promote the formation of ligamentous tissue. Several groups have reported on potential ACL scaffolds using collagen, silk, biodegradable polymers, and composite materials [1,5,19–23]. Our approach to the design of functional ACL replacement grafts focuses on several parameters: architecture, porosity, degradability, and cell source. A strong emphasis is placed on understanding the effects of varying these three design parameters on the overall mechanical properties and cellular response to the tissue-engineered scaffold.

The architecture of the tissue-engineered scaffold is an important design consideration that can modulate biological response and long-term clinical success of the scaffold. It has been reported that calcified tissue ingrowth can occur at a minimum pore size of 100 μm [24]. In addition, a minimum pore diameter of 150 μm is suggested for bone and 200–250 μm for soft tissue ingrowth [11,25,26]. Scaffolds developed within these pore size ranges will encourage tissue ingrowth, capillary supply, and improve the quality of anchorage in bone tunnels. Overall scaffold porosity can modulate the functionality and gross cellular response to the implant. The presence of pore interconnectivity extending through an implant increases the overall surface area for cell attachment, which in turn can enhance the regenerative properties of the implant by allowing tissue ingrowth into the interior of the matrix.

The FDA has approved the use of the poly-α-hydroxyesters [polylactic acid (PLA), polyglycolic acid (PGA) and copolymers, polylactide-*co*-glycolide (PLAGA)] for a variety of clinical applications, and they have been investigated for use in tissue engineering [1,18,27–29]. The growing emphasis on the use of biodegradable materials is due to the fact that these materials do not elicit a permanent foreign body reaction, as they are gradually reabsorbed and replaced by natural tissue. In the long term, fatigue properties of the material may be less of a concern as the scaffold is eventually replaced by natural tissue. Therefore, PLAGA fibers, due to their well-documented biocompatibility, biodegradability, and extended clinical use as sutures and fixations devices, were chosen for study as part of a tissue-engineered scaffold.

The native ACL consists of a large number of fiber bundles arranged into three areas: anteromedial, posterolateral, and intermedial, accommodating low levels of friction tension during a wide range of motion [30,31]. By mimicking the collagen fiber matrix of the natural ACL, our approach was to engineer functional ACL scaffolds based on three-dimensional (3-D) fibrous hierarchical designs, utilizing novel braiding techniques which permit controlled fabrication of substrates with a desired pore diameter, porosity, mechanical properties, and geometry. The objective was to design a scaffold that would provide the newly regenerating tissue a temporary site for cell attachment, proliferation, and mechanical stability.

In addition to scaffold architecture and degradability, cell source and cellular response are also important consideration in ACL tissue engineering. Primary ACL fibroblasts derived from either explant or digestive cultures have a lower doubling rate compared to cells from other soft tissues. For in vitro culturing, rapid cell growth and maturation is desired in order to lower the wait time between cell harvesting and graft incorporation, which may be particularly important from a therapeutic standpoint. Therefore, other cell sources have been considered for ACL tissue engineering [32]. In this study, we performed an in vitro assessment of scaffold biocompatibility, where cell attachment, growth, and long-term matrix elaboration by primary ACL cells were compared to those of a murine fibroblast line. The primary criteria for cell selection were based on whether the alternative cell source can reproduce or mimic the response of native ACL cells when exposed to the designed replacement scaffold.

2. Materials and methods

2.1. Scaffold fabrication

The 3-D fibrous scaffolds were fabricated using customized, 3-D circular and rectangular braiding machines [33–35]. PLAGA 10:90 (Ethicon, NJ) fibers (52 deniers) were laced to produce yarns with a yarn density of 18 yarns per yarn bundle. The PLAGA yarns were then placed in a custom built circular braiding loom with a 3×16 carrier arrangement. The circular braiding machine uses the sequential motion of the carriers (alternating tracks) to form 48-yarn, 3-D circular braids with braiding angles that ranged from 26° to 31°. The scaffolds measured 2 cm in length for the porosity studies. For comparison in architecture and as an alternative design, the scaffolds were also fabricated using a 3-D rectangular braiding system in which PLAGA fibers were laced to produce yarns with yarn densities of 9, 30, and 60 yarns per bundle to investigate effects on mechanical and porosity parameters due to fiber number.

2.2. Scaffold characterization

The as-made scaffolds were characterized in terms of architecture (pore diameter, porosity, surface area), and