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The use of layer by layer self-assembled coatings of hyaluronic acid and cationized gelatin to improve the biocompatibility of poly(ethylene terephthalate) artificial ligaments for reconstruction of the anterior cruciate ligament

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ABSTRACT

In this study layer by layer (LBL) self-assembled coatings of hyaluronic acid (HA) and cationized gelatin (CG) were used to modify polyethylene terephthalate (PET) artificial ligament grafts. Changes in the surface properties were characterized by scanning electron microscopy, attenuated total reflection Fourier transform infrared spectroscopy, energy-dispersive X-ray spectroscopy, and contact angle and biomechanical measurements. The cell compatibility of this HA–CG coating was investigated in vitro on PET films seeded with human foreskin dermal fibroblasts over 7 days. The results of our in vitro studies demonstrated that the HA–CG coating significantly enhanced cell adhesion, facilitated cell growth, and suppressed the expression of inflammation-related genes relative to a pure PET graft. Furthermore, rabbit and porcine anterior cruciate ligament reconstruction models were used to evaluate the effect of this LBL coating in vivo. The animal experiment results proved that this LBL coating significantly inhibited inflammatory cell infiltration and promoted new ligament tissue regeneration among the graft fibers. In addition, the formation of type I collagen in the HA–CG coating group was much higher than in the control group. Based on these results we conclude that PET grafts coated with HA–CG have considerable potential as substitutes for ligament reconstruction.

1. Introduction

Damage to the anterior cruciate ligament (ACL) is one of the most common sports injuries. It has limited ability to heal itself after it is ruptured by trauma [1]. The main treatment for ACL injury has been to reconstruct the ACL using autograft or allograft tendon [2]. However, autograft harvest can result in pain, weakness and altered biomechanics and morbidity at the harvest site, while allograft implantation can result in disease transmission, infection, and allergic reactions [3]. Because of these drawbacks there is significant interest in the use of artificial ligaments in ACL reconstruction.

The Ligament Advanced Reinforcement System (LARS) is one such artificial ligament [4]. This type of artificial ligament is non-degradable and maintains good mechanical durability. Several investigations have reported that LARS artificial ligaments produce safe and satisfactory results in the reconstruction of knee ligaments [5–8]. However, some failure cases have been noted [9–11]. Guidoin et al. [9] analyzed 117 ACL prostheses excised after they had ruptured and developed synovitis after implantation. Chronic inflammatory reactions were observed, with macrophages and giant cells in the polyester ligaments. Poorly organized and unpredictably distributed collagen tissue, which resembled scar tissue, infiltrated between the fibers during healing, which may induce loss of integrity of the ligament textile structure and result in failure of the artificial ligament. Recently a rare case of serious synovitis was reported in a 26-year-old man with a LARS artificial ligament reconstruction. The 3-year post-operative observations revealed thick fibrous scar tissue around the graft and poorly organized fibrous scar tissue between the graft fibers, which might cause a loss of structural integrity of the ligament and eventual failure of the graft [10]. These failures indicated that the polyethylene terephthalate (PET) artificial ligament graft had poor "ligamentization" in the knee joint after implantation [12].

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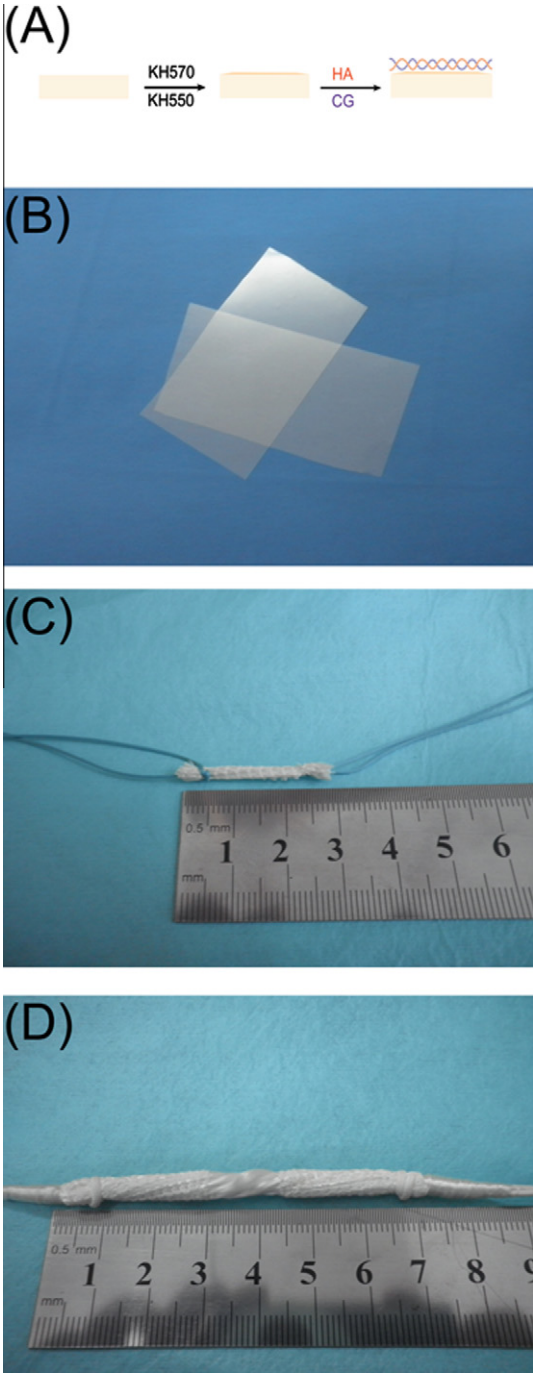

Fig. 1. (A) Schematic diagram of surface modification process; (B) PET films; (C) PET graft; and (D) PET ligament. PET, polyethylene terephthalate.

In general a reconstructed graft will incorporate itself into a new environment and transform itself into a structure similar to the normal ACL (the "ligamentization" process). This process consists of four phases: initial avascular necrosis, revascularization, cellular repopulation and, finally, remodeling with collagen orientation [13]. After implantation the surface characteristics of the graft strongly influence the attachment and growth of cells on ligament fibers. Rough surfaces with intermediate hydrophilicity, a positive charge and high surface energy are favorable for cell attachment and growth [14]. The LARS ligaments are made of PET fibers. This material is hydrophobic and chemically inert and has poor biological properties, which are unfavorable for cell adhesion and proliferation, revascularization, collagen synthesis, fiber coverage with extracellular matrix (ECM), and ligament regeneration [15]. These drawbacks have prompted ongoing research to modify the surfaces of PET artificial ligament grafts to improve their biocompatibility and enhance their ligamentization in vivo.

Table 1
Primer sequences.

Gene	Sequences
IL1	Forward: TGCTGGTTCCCTGCCCACAGA
	Reverse: TCCCGGAGCGTGCAGTTCAGT
TNF-α	Forward: CTCCAGTGGCTGAACCGCCG
	Reverse: AGCACATGGGTGGAGGGGCA
MMP1	Forward: AGACAAAGGCAAGTTGAAAAGCGGA
	Reverse: TTGCTCCCAGCGAGGGTTCC
MMP3	Forward: ACAAGGAGGCAGGCAAGACAGC
	Reverse: GCTGAGCAAACTGCCACGCA
CXCL2	Forward: TGTCTCAACCCCGCATCGC
	Reverse: CTTCAGGAACAGCCACCAATAAGC
CXCL8	Forward: TTGGCAGCCTTCCTGATTT
	Reverse: GGGTGGAAAGGTTTGGAGTAT
GAPDH	Forward: GTGGACCTGACCTGCCGTCT
	Reverse: GGAGGAGTGGGTGTCGCTGT

In the present study we have tried to improve the ligamentization of artificial ligaments by coating the graft surface with biocompatible, cytophilic and hydrophilic natural polymers. Hyaluronic acid (HA) and cationized gelatin (CG) were chosen for their oppositely charged polymer electrolyte characteristics to form a surface coating through a layer by layer (LBL) self-assembly method. The LBL self-assembly technique, which takes advantage of static or hydrogen bond interactions between different kinds of macromolecules, has emerged as a useful and versatile method for the modification of biological and non-biological templates for various biomedical applications [16–18]. HA has been utilized to make artificial grafts a more desirable biomaterial scaffold [15,19], although HA alone does not support cell attachment and spreading and needs chemical modification to support cellular adhesion. Previously Yamanlar et al. [20] applied the LBL technique to functionalize photocrosslinked HA hydrogels by deposition of poly(L-lysine) (PLL) and HA multilayer films. An in vitro study using NIH 3T3 fibroblasts showed improved cell attachment and spreading on the multilayer functionalized hydrogels. Funakoshi et al. [19] investigated the feasibility of using novel chitosan-based HA hybrid polymer fibers as a scaffold in ligament tissue engineering, and found that they enhanced rabbit fibroblast adhesion and type I collagen production. Recently Cado et al. [21] also reported that the chitosan/HA system appears to be best for cell adhesion, inducing the clustering of CD44, a cell surface HA receptor, in the membrane of cells. CG is a positively charged polymer with a high charge density in solution. It is a valuable carrier system [22] and effectively enhances the affinity of polymers for cells [14]. It has also been reported that electrospun poly(lactic acid) (PLLA) nanofibers modified with CG has improved compatibility with chondrocytes and CG-grafted PLLA nanofibrous membranes showed potential application as a cartilage tissue engineering scaffold [23].

In this study we have tried to apply HA–CG multilayers to the surface of a PET ligament graft through an LBL self-assembly process and evaluated the efficacy and feasibility of HA–CG coatings for the improvement of graft biocompatibility in vitro and in vivo. We hypothesized that the HA–CG coating had a positive effect in promoting the graft biocompatibility and enhancing the ligamentization of grafts after implantation.

2. Materials and methods

2.1. Preparation of cationized gelatin

4.0 g of ethylenediamine (Sinopharm Chemical Reagent Co.) and 2.0 g of N′-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydro-

ID	Title	Pages
378	The use of layer by layer self-assembled coatings of hyaluronic acid and cationized gelatin to improve the biocompatibility of poly(ethylene terephthalate) artificial ligaments for reconstruction of the anterior cruciate ligament	13