Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 26 (2005) 6635–6642
Biomaterials
www.elsevier.com/locate/biomaterials

Adhesion strength of human tenocytes to extracellular matrix component-modified poly(DL-lactide-*co*-glycolide) substrates

Ting-Wu Qin[a,*], Zhi-Ming Yang[a], Ze-Zhi Wu[b], Hui-Qi Xie[a], Jian Qin[b], Shao-Xi Cai[b]

[a]*Institute of Stem Cell and Tissue Engineering, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, People's Republic of China*
[b]*Bioengineering College, Chongqing University, Chongqing 400041, People's Republic of China*

Received 18 December 2004; accepted 12 April 2005
Available online 6 June 2005

Abstract

We report a direct measurement of the adhesion strength of human embryonic tenocytes (HETCs) and transformed human embryonic tenocytes (THETCs) to fibronectin (FN)- and type I collagen (CNI)- modified poly(DL-lactide-*co*-glycolide) (PLGA) substrates with a micropipette aspiration technique. PLGA substrates were first coated with poly-D-lysine (PDL), and then with various concentrations (1 μg/ml, 2 μg/ml, 5 μg/ml, and 10 μg/ml) of FN and CNI in serum-free F12 media. Anti-FN and Anti-CNI antibodies were used to inhibit attachment of tenocytes to FN- and CNI- modified substrates in a dilution range of 1:5000–1:500 and 1:1500–1:250, respectively. The substrates were employed for incubation of HETCs and THETCs for 30 min at 37 °C before the adhesion strength measurements. We found that the adhesion strengths showed a strong dependence on the seeding time and FN or CNI concentrations. Anti-FN and Anti-CNI antibodies significantly compromised adhesion of HETCs and THETCs to the corresponding modified substrates ($P<0.05$). These findings show that FN- or CNI-modified polymer substrates offer significant advantages for tissue engineering tendon scaffolds concerning tenocyte adhesion. In addition, HETCs and THETCs bear similar biological behaviors in terms of adhesion, indicating the possibility of using THETCs in place of HETCs in tissue engineering construction of human tendons.

Keywords: Adhesion strength; Tenocyte; Fibronectin; Collagen; Poly(DL-lactide-*co*-glycolide)

1. Introduction

The adhesion of tenocytes to extracellular matrix (ECM) plays a critical role in tissue engineering tendons, as successful tendon genesis entails the ability of tenocytes to adhere to an ECM scaffold, proliferate, and finally organize ECM molecules into a functional tendon [1]. Many efforts have been focused on improving cell adhesion properties to various scaffolds while reconstructing the tissue engineering tendons. These scaffolds may be either natural or synthetic such as naturally derived materials [2–5], carbon fibers [6], and polymers [7,8]. The scaffolds mimic the ECM by providing structural components that can physically support tendon regeneration. Degradable polymer scaffolds may provide temporary support for tenocytes during vascularization and formation of a functional tendon. However, their surfaces usually do not provide for efficient cell attachment and growth. Desirable features of these scaffolds would be the provision of biosignaling molecules on their surfaces, such as cell adhesion proteins [9,10], growth factors [11], or cytokines that may be critical for proper cellular function. Poly(DL-lactide-*co*-glycolide) (PLGA) scaffolds have been used in a cell-material system for guided tenocyte growth [12]. However, these scaffolds are hydrophobic, and they are desired to impart hydrophilicity to these polymers by introducing adhesion proteins.

*Corresponding author. Tel./fax: +86 28 85422083.
E-mail address: qtw@mcwcums.com (T.-W. Qin).

0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.04.023

The adhesion characteristics of tenocytes depend on both the expression of cell adhesion proteins and their interaction with the ECM. Fibronectin (FN) and type I collagen (CNI) are two major ECM components in tendons [13]. They mediate cell attachment and provide substrates for cell growth in regenerative tendons [14–16], stimulating cell migration and differentiation [17,18]. Conditioning or coating of tissue engineering scaffolds with ECM components usually aims at enhancing tenocyte adhesion and proliferation. This makes the understanding of tenocyte adhesion behaviors to scaffolds even more crucial in three-dimensional tissue assembly. However, to the best of our knowledge, quantitatively, few reports focused on the strengths of tenocyte adhesion to ECM scaffolds.

The aim of this study was to determine the adhesive strengths of human embryonic tenocytes (HETCs) and transformed human embryonic tenocytes (THETCs) to PLGA substrates coated with ECM components, and compare the differences between the responses of the two types of tenocytes to these substrates. We focused on the effects of seeding time, which allow the tenocyte adhesion to be established, polymer surface, FN and CNI coating concentrations, and antibody blocking, on the tenocyte adhesion strength. FN and CNI induced integrin-mediated interactions, which can be blocked by specific antibodies. Our results indicated that the adhesion strengths of tenocytes to ECM component-modified substrates had a good dependence on the seeding time and the coating concentrations. Also, the antibodies to FN and CNI inhibited the adhesion of tenocytes to substrates. No significant differences in adhesion strengths were shown between HETCs and THETCs.

2. Materials and methods

2.1. Cell culture

HETCs were isolated via sequential trypsin digestions of human embryo tendon of spontaneous abortion according to the established protocol [19]. The cryopreserved THETCs were rewarmed. HETCs and THETCs were cultured with F12 media (Gibic Co.) supplemented with 10% fetal bovine serum (FBS), 100 μg/ml penicillin, 100 μg/ml streptomycin were separately cultured in a humidified air with 5% CO_2. Upon about 70% confluence in tissue culture dishes (Falcon Labware), HETCs (passage 2–4) and THETCs (passage 39–42) were trypsinized with 0.25% trypsin in F12 media for 2 min, washed two times with the culture media, and then stored in F12 incomplete media (without FBS) until cell seeding.

2.2. Preparation of polymer films and protein-modified substrates

PLGA (75:25) polymer (average MW 113 200, Sigma Chemical Co., the ratio 75:25 designating the copolymer ratio of lactide to glycolide) was used to prepare polymer films. In 10 ml chloroform 300 mg PLGA was dissolved. About 300 μl of the polymer solution was placed on the bottom surface of a micropipette chamber (described in Section 2.3). The solvent was allowed to evaporate for 24 h at room temperature. The films (0.1–0.3 mm thick) were subsequently coated with 5 μg/ml poly-D-lysine (PDL) (MW 220 000, Sigma Chemical Co.) at 37 °C for 30 min. In order to form the protein-modified substrates, the films were washed with PBS to remove excessive PDL and then coated with various concentrations (1, 2, 5, and 10 μg/ml) of FN (Sigma Chemical Co.) or CNI (Sigma Chemical Co.) in serum-free F12 media. After 30 min of incubation at 37 °C, the substrates were washed by F12 incomplete media. These substrates were sterilized by ultra-violet, followed by rinsing in sterile PBS. Tenocytes in the F12 incomplete media were carefully seeded on the substrates in the chamber at a density of 5×10^4 cells/ml and then incubated for 30 min at 37 °C before the micropipette experiment.

2.3. Measurement of adhesion strengths

The method used for quantifying adhesion strengths was the micropipette aspiration technique [20]. Micropipettes with an internal radius of 2.5–3.3 μm were prepared with a micropipette puller (Model P-87, Sutter Instrument Co., Novato, CA), and then filled with the culture media and mounted on a hydraulic micromanipulator with the wide end of the pipette connected to a pressure recording system. The micropipette chamber consisted of a sectioned plastic cylinder of about 2 ml in volume and 15 mm in diameter was glued to a cover glass. A breach was made at the sidewall of the cylinder to make the micropipette accessible to the inside of the chamber (Fig. 1). Micropipette manipulation was conducted under direct microscopic observation and recorded on a video recording system. The micropipette was manipulated to aspirate a small portion of the tenocyte by using a small aspiration pressure and then to pull the cell away from the adhering substrate. By step increments of the negative pressure, a critical pressure can be obtained to detach single cells away from the substrate (Fig. 2). Cell adhesion force was defined as the product of critical aspiration pressure and the pipette tip cross-sectional area, and then used as a measure of cell adhesion strength.

2.4. Effect of substrate modifications on adhesion strengths

Seeding time varied from 30 to 180 min. Tenocyte adhesion strengths to PLGA substrates were measured within 90 min post seeding. For four different concentrations of FN or CNI (1, 2, 5, and 10 μg/ml) on substrates, adhesion strength measurements were conducted with a fixed seeding time (90 min). Additionally, PLGA films were coated with 5 μg/ml of FN or CNI and incubated with anti-fibronectin antibody (Anti-FN, Sigma Chemical Co.) solution or anti-type I CNI antibody (Anti-CNI, Sigma Chemical Co) solution before tenocyte seeding. There were four different dilutions of Anti-FN (1:5000, 1:2500, 1:1000, and 1:500) and Anti-CNI (1:1500, 1:1000, 1:500, and 1:250). Addition of antibodies was to inhibit attachment of tenocytes to surface coated with FN or CNI. The control PLGA films were coated with 5 μg/ml of FN or CNI alone (without antibody solutions).