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SCIENCE DIRECT
Biomaterials 27 (2006) 3154–3162
Biomaterials
www.elsevier.com/locate/biomaterials

Combination of baculovirus-mediated gene transfer and rotating-shaft bioreactor for cartilage tissue engineering

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Received 30 September 2005; accepted 13 January 2006
Available online 2 February 2006

Abstract

We have previously demonstrated efficient baculovirus transduction of rat chondrocytes in 6-well plates. To further explore the potential of baculovirus in cartilage tissue engineering, the baculovirus-transduced chondrocytes were seeded into porous scaffolds and cultivated in a rotating-shaft bioreactor (RSB) which was developed for two-phase cultivation of tissue engineered cartilage. The baculovirus transduction resulted in efficiencies up to 90%, and affected neither cell adhesion to the scaffolds nor cell survival in the RSB. After 4-week RSB cultivation, the transduced cells remained highly differentiated and grew into constructs that resembled the untransduced constructs with regard to gross appearance, construct size, cell morphology, cell spatial distribution, glycosaminoglycan and collagen production and deposition. Importantly, baculovirus transduction did not alter the expression of chondrocytic genes. These data confirmed that baculovirus transduction neither harms chondrocytes nor retards the formation of cartilage-like tissues in the RSB, thus implicating the potentials of combining baculovirus-mediated gene transfer with RSB cultivation in in vitro cartilage tissue engineering.

Keywords: Baculovirus; Bioreactor; Cartilage; Chondrocyte; Gene therapy; Tissue engineering

1. Introduction

Healthy articular cartilage consists of chondrocytes embedded in the extracellular matrix (ECM) in which chondrocytes synthesize matrix molecules such as collagen II and glycosaminoglycans (GAGs) to maintain the structural integrity and desirable mechanical properties. However, the aneural and avascular nature of articular cartilage limits its ability to self-repair upon the occurrence of trauma or joint diseases. Meanwhile, surgical implantation is limited by the availability of donor tissues. Consequently, cartilage tissue engineering has been motivated by the need to create implantable tissue equivalents for clinical use [1]. One common tissue engineering approach is to immobilize appropriate cells (e.g. chondrocytes) in the scaffolds and cultivate the cell/scaffold constructs in a bioreactor for tissue growth prior to implantation (for review, see [2]). To meet this demand, we have previously developed a rotating-shaft bioreactor (RSB) for two-phase cultivation of tissue engineered cartilage [3]. The RSB cultivation of cell/scaffold constructs for 4 weeks resulted in uniform cell distribution, increased GAG and collagen deposition, and formation of cartilage-like tissues.

An alternative strategy emerging in recent years involves the combination of cell therapy and gene transfer techniques. For instance, retroviral vector expressing TGF-β1 is employed to transduce chondrocytes to maintain chondrocyte re-differentiation [4]. Adenoviral vector and plasmid DNA expressing IGF-1 are utilized for ex vivo gene delivery to chondrocytes [5,6]. With this approach, the genetically modified cells are transplanted into animal models so that the expressed factors can promote tissue regeneration in vivo.

Baculovirus (*Autographa californica* nuclear polyhedrosis virus, AcMNPV) has been widely employed for recombinant protein production in insect cells. Since the finding that baculovirus is capable of transducing mammalian cells in 1995, increasing efforts have been directed

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0142-9612/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2006.01.018

toward developing baculovirus as a gene delivery vector for in vitro and in vivo gene therapy studies [7,8]. Given the potentials of chondrocytes for cartilage tissue engineering and baculovirus for gene delivery, we have demonstrated, for the first time, that baculovirus efficiently transduce primary rat chondrocytes cultured on 6-well plates [9]. However, how baculovirus-mediated transgene expression affects cell survival and differentiation in the 3-dimensional (3D) environment and whether baculovirus transduction hinders cartilage formation remain unknown. To exploit baculovirus as a gene delivery vector for cartilage tissue engineering, in this study, we attempted to answer the above questions by transducing chondrocytes with a baculovirus expressing enhanced green fluorescent protein (EGFP) as the model protein, and subsequently cultivating the transduced chondrocytes in the RSB that mimics the dynamic 3D in vivo environment. The transgene expression, ECM production and chondrocyte-specific gene expression by the transduced chondrocytes cultivated in the RSB were evaluated. The significances of the present work are discussed.

2. Materials and methods

2.1. Virus preparation

The recombinant baculovirus expressing EGFP under the control of cytomegalovirus immediate-early (CMV-IE) promoter was constructed previously and designated Bac-CE [10]. The virus was amplified by infecting Sf-9 cells grown in TNM-FH medium (Sigma) containing 10% fetal bovine serum (FBS, Sigma). Virus supernatant was harvested at 4 days post-infection and stored at 4 °C without concentration. Virus titers were determined using the endpoint dilution method [11] and ranged between 5×10^8 and 4×10^9 plaque forming units per milliliter.

2.2. Chondrocyte culture and virus transduction

Primary chondrocytes were isolated from the articular cartilages of 7-day-old Wistar rats as described previously [9]. The cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% FBS and 50 μg/ml ascorbic acid at 37 °C, and passaged once (P1) for subsequent transduction unless otherwise noted. The chondrocytes were transduced according to an optimized protocol developed recently [12]. In brief, the cells were plated onto 6-well plates or T-175 flasks at 50% confluency and incubated overnight. The medium was removed prior to transduction, and the cells were washed 3 times by Dulbecco's phosphate buffered-saline (PBS). The transduction was initiated by adding 0.1 ml (6-well plates) or 2 ml (T-175 flasks) unconcentrated virus solution, followed by adding 0.4 ml (6-well plates) or 8 ml (T-175 flasks) PBS as the surrounding solution. Then the cells were shaken on the rocking plate for different periods of time (2, 4 or 8 h) at 25 °C. After the virus incubation period, virus solution was replaced by fresh medium and the culture continued. For 2D monolayer culture, medium change was performed twice a week. For 3D cultivation, the cells were trypsinized at 1 day post-transduction (dpt) and seeded into scaffolds (see below).

2.3. Three-dimensional cultivation in the RSB

The design and operation of RSB have been described previously [3]. The main body of RSB comprised a water-jacketed glass cylinder (inner diameter = 5 cm, height = 10 cm), a sealing cap assembly and a stainless shaft on which 22-gauge needles were soldered (Fig. 1). The RSB was oriented horizontally and the rotation of the shaft was driven by a low-speed, bi-directional peristaltic pump. Ports at both ends of the reactor were connected to a 5% CO_2 incubator and an external medium reservoir (500 ml), respectively, for gas and medium perfusion. The temperature was controlled at 37 °C by the water circulating through the water jacket and an external water bath.

For 3D cultivation, the chondrocytes were dynamically seeded into the poly(L-lactide-*co*-glycolide) (PLGA, Purac Inc., Netherlands) scaffolds. The porous PLGA scaffolds were fabricated to disk shape (≈7.5 mm diameter, ≈3.5 mm thickness) using an established solvent merging/particulate leaching method [13], positioned on 8-cm-long, 22-gauge needles (four needles with two scaffolds each), and suspended in a 100 ml spinner flask containing 75 ml culture medium (DMEM supplemented

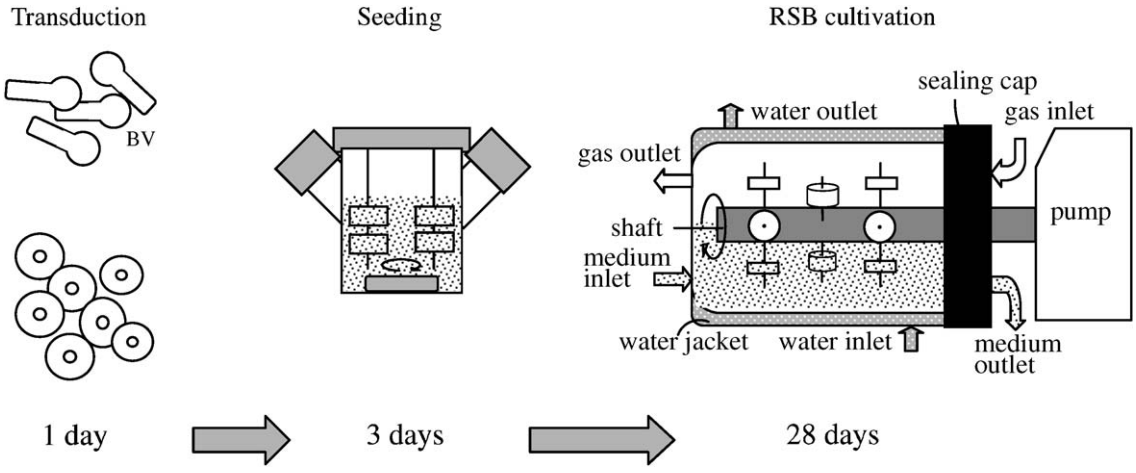

Fig. 1. Schematic illustration of the transduction, seeding and 3D cultivation of chondrocytes. The primary rat chondrocytes were isolated, passaged once (P1), plated on T-150 flasks overnight and transduced with 2 ml virus solution and 8 ml PBS for different periods of time (2, 4 and 8 h). At 1 dpt, the cells were trypsinized and dynamically seeded into PLGA scaffolds in the spinner flasks. After 3 days, the cell/scaffold constructs were transferred and positioned on the needles soldered to the RSB shaft, then half of the reactor space was filled with medium (≈100 ml). The RSB was oriented horizontally and operated in a perfusion mode (medium flow rate = 0.2 ml/min, gas flow rate = 20 ml/min) with the shaft rotation speed maintained at 10 rpm in the first 3 weeks and at 2 rpm in the 4th week.