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Dynamic compression and co-culture with nucleus pulposus cells promotes proliferation and differentiation of adipose-derived mesenchymal stem cells

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ABSTRACT

Adipose-derived stem cells (ASCs) are a set of multi potent stem cells potentially used in cartilage tissue engineering. We hypothesized that the effect of dynamic compression and co-culture with nucleus pulposus cells (NPCs) promotes ASC proliferation and chondrogenic differentiation. A controlled dynamic compression loading device was utilized to stimulate ASCs obtained from Sprague Dawley (SD) rats and identified by flow cytometry. The proliferation index was measured by carboxyfluorescein succinimidyl ester (CFSE) staining. Dynamic compression, as well as co-culture enhanced chondrogenic differentiation of ASCs as indicated by the expression of SOX-9, type-II collagen and aggrecan, which were measured by real-time PCR and Western blot. In our study, we found dynamic compression promoted the proliferation of ASCs and induced its differentiation into NP-like cells. Combination of dynamic compression and co-culture showed an additive effect on NP-like cell differentiation.

1. Introduction

Intervertebral disc degeneration (IDD) entails disc herniation, characterized by progressive loss of proteoglycans and fibrosis of the nucleus pulposus cells (NPCs). Lack of effective surgical or therapeutic intervention has been a bottleneck in IDD treatment. Current cellular therapies have been tested in animal models showed limited success in ameliorating degenerative disc disease (Sobajima et al., 2008). Clinical trials show reduced pain in patients treated with disc chondrocyte transplantation (Meisel et al., 2007). Tissue and cellular engineering approaches are promising to restore the structure and function of intervertebral disc and further delay the progression of IDD. Insertion of NPCs reportedly had diminished additional disc degeneration in an animal model (Nishimura and Mochida, 1998; Okuma et al., 2000). However, autologous approaches are characterized by limitations of availability in the degenerated tissue as well as limited number of chondrocytes (only 5–10%) in the cartilage tissue (Chung and Burdick, 2008). Thus, the need for alternate solutions cannot be overstated.

Mesenchymal stem cells (MSCs) are a set of stem cells carrying multi-directional differentiation potentiality. Converging evidence demonstrated that bone marrow mesenchymal stem cells (BMSCs) differentiated into NP-like cells when co-cultured with NPCs (Niu et al., 2009; Richardson et al., 2006). Co-culture with NPCs significantly induced chondrogenic differentiation of MSCs, characterized by upregulated expression of collagen type II, aggrecan and SOX-9 (Niu et al., 2009; Richardson et al., 2006). Currently, the feasibility of the co-culture approach has been investigated to support stem cell therapy for IDD (Sobajima et al., 2008). Although the specific induction mechanism remains elusive, it is rational to speculate that the co-culturing approach leads to production of sufficient NPCs for IDD treatment.

More recently, adipose-derived mesenchymal stem cells (ASCs) with significant chondrogenic potential (Guilak et al., 2006) were obtained. However, access to large amounts is a challenge despite inexpensive and robust strategies for differentiation of ASCs into chondrocytes in a reproducible and highly efficient manner (Peran et al., 2013; Diekman et al., 2010; Estes et al., 2010). ASCs have been demonstrated to display a cytokine profile similar to MSCs (Kilroy et al., 2007). Understanding of the development, growth, and physiology of native cartilage enables successful induction of chondrogenic differentiation and regeneration clinically. Chondrocytes sense the hydrostatic pressure (HP), which partially simulates the disc environment. HP can be used with chondrocytes in monolayer, three-dimensional (3-D) engineered constructs, as well as explants (Elder and Athanasiou, 2009). It has been confirmed that external environment plays a critical role in promoting cell growth and differentiation. Mechanical signals result in altered intracellular ion concentrations, which may

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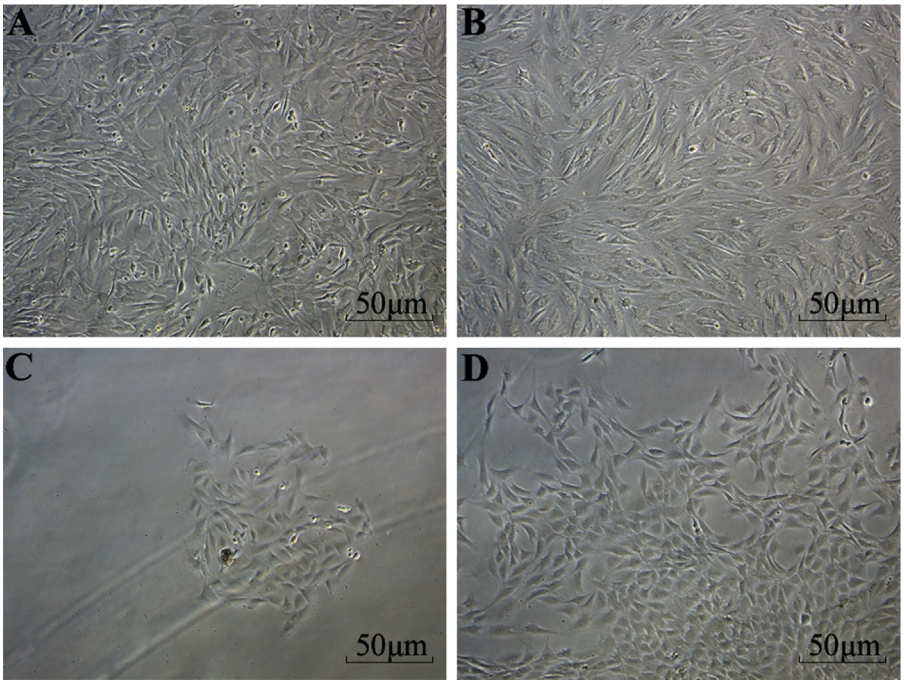

Fig. 1. Culture of ASCs and NPCs. (A) The primary ASCs were thawed after 24 h; (B) The third generation of ASCs cultured in monolayer, under inverted phase contrast microscope; (C) NPCs cultured for 48 h; (D) NPCs cultured for 7 days.

affect gene and protein expression, and hence, the biomechanical properties of cartilage tissue (Safshekan et al., 2012).

Dynamic compression using HP has been regarded as a key factor in regulating the proliferation and differentiation of MSCs and ASCs. Appropriate compressive stress promotes the chondrogenic differentiation of BMSCs concomitant with enhanced gene expression of aggrecan and collagen II (Huang et al., 2004; Meyer et al., 2011). Furthermore, the synergistic interaction between dynamic compression and TGF-β signaling pathway resulted in chondrogenic differentiation of MSCs, which underscored the importance of combining mechanical stimulation with other factors (Mouw et al., 2007). Another study demonstrated that cyclic hydrostatic pressure loading enhanced expression of genes associated with chondrogenic differentiation of ASCs (Ogawa et al., 2009), pointing toward the roles of mechanical stimuli in the induction of chondrogenic differentiation of ASCs. However, the interaction of mechanical stimuli and co-culture system on the proliferation and differentiation of ASCs remains unclear. Therefore, in this study, we investigated the interactive effects of dynamic compression and co-culture with NPCs on the proliferation and chondrogenic differentiation of ASCs.

2. Materials and methods

2.1. ASCs and NPCs cultures

Following approval by the Institutional Animal Care and Use Committee of Tongji Medical College of HUST, lumbar intervertebral disc (nucleus pulposus) tissue was obtained from 20 Sprague Dawley rats (aged 4 weeks, and weighing 200 g each) and harvested under aseptic conditions. The tissue was washed twice by PBS and cut into small explants of about $1 \times 1 \times 1$ mm^3. Tissue explants were evenly distributed over the surface of a 5-mL disposable flask pretreated with culture medium. We replaced the medium initially when the NPCs showed an adhesive growth after 48 h, and then continuously for 3 days. These cells were maintained in DMEM/F12 medium and cultured at 37 °C with 5% CO_2.

We used adipose tissue from bilateral groins of the same 20 rats to isolate and obtain ASCs simultaneously, as previously described (Zuk et al., 2001). The ASCs were maintained in DMEM/F12 supplemented with 10% heat-inactivated fetal bovine serum (Gibco, USA) containing 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco, USA) and incubated at 37 °C and 5% CO_2.

2.2. Flow cytometry

Briefly, ASCs were digested by EDTA-trypsin and resuspended in PBS at a final concentration of 1×10^6 cells/ml, and then incubated with FITC-conjugated antibody (CD29, CD44, CD34 and CD45, Santa Cruz, USA) for 30 min at room temperature. After washing twice by PBS, cells were analyzed by FACSCalibur (Becton Dickinson, USA).

2.3. Co-culture and dynamic compression

Alginate beads were used to package the NPCs for separation from ASCs in T-25 culture flasks. NPCs were harvested from T-25 flask after 48-h culture and encapsulated in alginate beads in individual wells of six-well plates. The ten alginate beads encapsulating NPCs were co-incubated with ASCs (approximately 1:1 ratio) in a T-25 flask at 37 °C and 5% CO_2 in DMEM/F12 with 10% heat-inactivated fetal bovine serum. Mechanical stimulation was achieved by a dynamic compression apparatus (Kim et al., 2009) shown in Supplementary data Fig. 1.

We controlled the flask pressure by adjusting the air pressure at both entrance and exit. An open pressure-adjusting system was created by placing the gas circulating system in the CO_2 incubator, including a pressure pump, control device, flow valve and an incubator (Fig. 3). We set the pressure pump to provide intermittent dynamic hydrostatic pressure at 17 kPa under 220 V. The apparatus operated on alternating 12-h shifts of work and rest. It allowed 4 days of proliferation and 7 days of differentiation. To further investigate the effects of dynamic compression and co-culture on cell differentiation, we divided the experiment into six groups in triple parallel. Single cultures of ASCs (group I) and NPCs (group II) were used as the controls. ASCs and NPCs cultured under dynamic compression were designated as group III and group IV, respectively. ASCs and NPCs co-cultured in the presence and absence of dynamic compression were set as group V and group VI, respectively (Table 1).

2.4. Carboxyfluorescein succinimidyl ester (CFSE) staining

ASCs were collected and washed twice by PBS, and stained with 5 μM CFSE (Cell Trace CFSE cell proliferation kit, Invitrogen, USA) for 15 min at 37 °C, according to the manufacturer's instructions. Cells were then seeded in culture flasks, and harvested at different times (24 h, 48 h, 72 h and 96 h). The mean fluorescence intensity (MFI) was measured in each case, using flow cytometry (Becton Dickinson, USA). Additionally, the stained cells were photographed under a fluorescence microscope (Nikon, Japan) (Fig. 4).