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Development of a cell culture system loading cyclic mechanical strain to chondrogenic cells

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Abstract

Mechanical stimulation is considered to be one of the major epigenetic factors regulating the metabolism, proliferation, survival and differentiation of cells in the skeletal tissues. It is generally accepted that the cytoskeleton can undergo remodeling in response to mechanical stimuli such as tensile strain or fluid flow. Mechanically induced cell deformation is one of the possible mechanotransduction pathways by which chondrocytes sense and respond to changes in their mechanical environment. Mechanical strain has a variety of effects on the structure and function of their cells in the skeletal tissues, such as chondrocytes, osteoblasts and fibroblasts. However, little is known about the effect of the quality and quantity of mechanical strain and the timing of mechanical loading on the differentiation of these cells. The present study was designed to investigate the effect of the deformation of chondrogenic cells, and cyclic compression using a newly developed culture device, by analyzing mechanobiological response to the differentiating chondrocytes. Cyclic compression between 0 and 22% strains, at 23 μHz was loaded on chondrogenic cell line ATDC5 by seeding in a mass mode on PDMS membrane, assuming direct transfer of cyclic deformation from the membrane to the cells at the same frequency. The compressive strain, induced within the membrane, was characterized based on the analysis of the finite element modeling (FEM). The results showed that the tensile strain inhibits the chondrogenic differentiation of ATDC5 cells, whereas the compressive strain enhances the chondrogenic differentiation, suggesting that the differentiation of the chondrogenic cells could be controlled by the amount and the mode of strain. In conclusion, we have developed a unique strain loading culture system to analyze the effect of various types of mechanical stimulation on various cellular activities.

Keywords: Mechanical strain; Mechanical stress; Chondrogenic cell; Culture system; Polydimethylsiloxane (PDMS); Finite element modeling (FEM)

1. Introduction

The musculoskeletal system is a unique combination of soft and hard tissues in the body, consisted of bone, cartilage, muscle and tendon. It has been well-documented that these skeletal tissue formations are in essence organized by the tissue specific cells, including osteoblasts, osteocytes, chondrocytes, myocytes, adipocytes and fibroblasts (Tondreau et al., 2004). Mesenchymal stem cells (MSCs) can be derived from bone marrow stroma and differentiate into multiple non-hematopoietic cell lineages such as those cells (Tondreau et al., 2004). Growth factors, cytokines, hormones, and other regulatory molecules are traditionally required in tissue engineering studies to direct the differentiation of MSCs along with specific lineages (Jiang

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et al., 2002). Previous studies have shown that physical factors, mechanical loading (Wong et al., 2003; Knight et al., 2006; Madhavan et al., 2006), electromagnetic fields (Aaron et al., 1999; De Mattei et al., 2004), and ultrasound (Naruse et al., 2000; Mukai et al., 2005), play an important role in regulating the function of MSCs. Recently, mechanical loading to the cells became to be considered as one of key regulators of the cellular metabolism (LaPlaca et al., 2005; Nicolella et al., 2006; Sato et al., 2007).

Mechanical loading is known to be one of important factors (Ruwhof and van der Laarse, 2000; De Croos et al., 2006). Cyclical strain activates the metabolism of bone forming cells, osteoblasts, and the mechanical compressive strain enhances the metabolism of cartilage specific extracellular matrix (ECM) aggrecan and type II collagen (Ruwhof and van der Laarse, 2000; De Croos et al., 2006). Thus, it is well established that the physiological mechanical loading is necessary for the development and maintenance of articular cartilage through the control of chondrocyte metabolism. The effects of mechanical strain on these cells are dependent on the magnitude (Honda et al., 2000), duration (Takahashi et al., 1998; Wall et al., 2007), and frequency (Knight et al., 1998; Pingguan-Murphy et al., 2005) of the mechanical strain. It has been widely proposed that dynamic mechanical conditioning may be utilized in vivo or within tissue engineering bioreactors to stimulate the production of the functional cartilage matrices (Wong and Carter, 2003; Seidel et al., 2004). Although multiple lines of evidence obtained in vitro and in vivo support the involvement of the mechanical strain to the cellular metabolism in the calcified tissue formation (Knight et al., 1998; Takahashi et al., 1998; Nicolella et al., 2005; Pingguan-Murphy et al., 2006; Sato et al., 2007), the cellular response to the quantitative loading of the mechanical strain, directly applied to the cells, has not been substantiated, regarding whether the amount mechanical strain is a regulating factor beyond the threshold of the stimulation. Moreover, the process to translate the mechanical strain into biochemical signals through which chondrocytes sense and respond to mechanical loading is not fully unidentified. The purpose of the present study is to investigate the efficacy of a newly developed culture system, with the quantitative mechanical strain, to the chondrogenic cell differentiation (Suzuki et al., 2003; Arai et al., 2005; Suzuki et al., 2005; Masuda et al., 2006). We examined the intracellular mechanobiological response of the differentiating chondrocytes by the mechanical strain.

2. Materials and methods

2.1. Design of cell culture system to load strain

A mechanical loading culture system containing patterned substrates was designed to induce the strain on chondrogenic cells. The mechanical strain loading culture system was composed of a polydimethylsiloxane (PDMS)-made chamber and a PDMS membrane. PDMS is widely used for the fabrication of various micro-devices because of its transparency, biocompatibility, remarkable advantage to induce mechanical properties, and low production cost. PDMS was mixed in 10:1 weight ratio (silicone rubber: curing agent). The solution was injected into the mold, a flame to formulate the culture device as a rigid chamber, and then cured at 60 °C for 4 h.

The PDMS chamber had the separate holes which were covered by a thin PDMS membrane (Fig. 1). These holes were connected with the tube (i.d. 1.2 mm) to decompress the space between the membrane and the hole. Thus, the PDMS membrane can be dented depending on the amount of decompression and the thickness of the membrane, due to the intrinsic elastic modulus. The PDMS chamber was designed in such a way that the cells inoculated on the PDMS membrane are quantitatively strained by utilizing the membrane deformation depending on the amount of decompression of the hole.

2.2. Application of mechanical strain and cell culture

A chondrogenic cell line, ATDC5, was obtained from RIKEN cell bank (Tsukuba, Japan). ATDC5 cells were maintained in DMEM/Ham's F12 (1:1) medium supplemented with 5% FBS, and antibiotics (100 U/ml 200 mM L-glutamine and 100 U/ml penicillin-G) at 37 °C in a humidified 5% CO_2 atmosphere. The PDMS membrane was coated with fibronectin (0.1% solution, from bovine plasma, SIGMA, St Louis, MO, USA) to obtain sufficient cell adhesion and the proliferation, after hydrophilic treatment of the membrane. To induce chondrogenesis, ATDC5 cell suspensions were spotted onto the PDMS membrane-attached culture system (hereafter referred to as PDMS plate) at a density of 5×10^6 cells/ml with 50 μl per separate PDMS plate and cultured in the above medium supplemented with ITS (10 μg/ml insulin, 5.5 μg/ml transferring, 6.7 ng/ml sodium selenite, GIBCO, Grand Island, NY, USA). After 3 h of pre-incubation, 5 ml of medium was added to each PDMS plate and maintained for 4 days with daily change.

This system is a computer-driven device that various mechanical strains can be conditioned, using vacuum pressure, to deform cells cultured on PDMS membrane. It was designed to be controlled by air actuator so as to not induce discontinuous strain when loading at a gradual signature curve speed and to avoid the sudden strain to the cells. First, single 22% local maximum strain was loaded in the cultures for 12 h, and then it was released and maintained for next for 12 h until re-loading. As illustrated in Fig. 1, chondrogenic cells were seeded on the membrane so as to cover enough the hole in PDMS plate (A). On the other hand, they were sown on the membrane within the hole in PDMS plate (B). In the present study, the PDMS membranes, having 100–600 μm in thickness, were used to produce different strain in the cell culture. The holes with 5 or 16 mm in diameter were made in the PDMS plates. The amount of the strain was analyzed by finite element modeling (FEM).

2.3. Finite element modeling (FEM) of the culture device

Using ANSYS 5.7, a 3D representation of the PDMS membrane and PDMS chamber has been used. The analysis models, assuming that the two surfaces were not slipped, integrated the