Journal of the Mechanical Behavior of Biomedical Materials 78 (2018) 404–413

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

A microfabricated platform for the study of chondrogenesis under different compressive loads

Reza Kowsari-Esfahan[a,b], Azadeh Jahanbakhsh[b], Mohammad Said Saidi[a,*], Shahin Bonakdar[b,*]

[a] School of Mechanical Engineering, Sharif University of Technology, Tehran 11155-9567, Iran
[b] National Cell Bank Department, Pasteur Institute of Iran, P.O. Box 13169-43551, Tehran, Iran

ARTICLE INFO

Keywords:
Microfluidics
Mechanobiology
Chondrogenic differentiation
Mechanical stimulus
Numerical simulation

ABSTRACT

Microfluidic devices are beneficial in miniaturizing and multiplexing various cellular assays in a single platform. Chondrogenesis is known to pertain to chemical, topographical, and mechanical cues in the microenvironment. Mechanical cues themselves have numerous parameters such as strain magnitude, frequency, and stimulation time. Effects of different strain magnitudes on the chondrogenic differentiation of adult stem cells have not been explored thoroughly. Here, a new multilayer microdevice is presented for the unidirectional compressive stimulation of cells in a three-dimensional cell culture. Numerical simulations were performed to evaluate and optimize the design. Results showed a favorable highly uniform axial strain distribution and negligible radial and circumferential strain for the optimized design. Moreover, an experimental study was performed on rabbit adipose-derived stem cells encapsulated *in-situ* in alginate hydrogel. Strain levels of 20%, 15%, 10%, 5%, and 0% were studied simultaneously on a microfluidic platform. Dynamic mechanical compression positively influenced cellular viability and upregulated collagen II, Sox-9, and aggrecan expression in the absence of exogenous growth factors. The expression of collagen type II as specific marker for articular chondrocytes was further confirmed by immunofluorescence staining of collagen type II. Taking together, 10% strain can be considered as optimal stimulation factor for chondrogenic differentiation of adipose derived stem cells.

1. Introduction

Cartilage tissue is known to have limited intrinsic regeneration, as the proliferation potency of mature chondrocytes is inconsiderable owing to its avascular nature (Mankin, 1982; Vinatier and Guicheux, 2016). Cartilage is susceptible to damage by direct and/or rotational trauma or repeated microtrauma (Brittberg, 2016). Damage can result in mobility disabilities and a painful experience for the patient (Brittberg, 2016) by degenerating the tissue, leading to osteoarthritis (Andriacchi and Mundermann, 2006). Osteoarthritis of the knee is one of the most prevalent non-fatal diseases affecting about 250 million of the world population (Vos et al., 2012) and its incidence is expected to increase substantially in future decades (Cross et al., 2014). Surgical methods to repair damaged cartilage such as abrasion arthroplasty lie in extrinsic marrow stimulation and are reported to result in a fibrocartilage tissue that cannot resemble the original hyaline cartilage (Dean et al., 2016), while the graft-based surgical technique can solve this problem (Chow et al., 2004).

Autograft and allograft transplantations can heal the cartilage lesion by maintaining hyaline characteristics of the native tissue (Chow et al., 2004; Dean et al., 2016). Thus, functional engineered cartilage constructs are highly demandable for tissue transplantation by supplying both viable chondrocyte and the matrix. Providing a large number of healthy chondrocytes from a patient or a donor for the coverage of large lesions is a challenge, considering the limited supply of cartilage (Musumeci et al., 2014) and challenges of *in-vitro* culture of chondrocytes (Panadero et al., 2016). Adipose-derived mesenchymal stem cells (ADSCs) with unlimited proliferation capacity, easy harvesting, and potency to differentiate to chondrocytes (Pittenger et al., 1999; Strem et al., 2005) can provide a precious source in the tissue engineering of cartilage. Mesenchymal stem cells can later be differentiated by sole chemical means (Kim et al., 2012; Kramer et al., 2000) or by modulation of substrate stiffness (Kwon, 2013) into functional chondrocytes. Chondrogenesis can also be done by a combination of chemical and mechanical (Bonzani et al., 2012; Terraciano et al., 2007) or topographical (Bonakdar et al., 2016) means.

While the physiological level of mechanical loading helps matrix biosynthesis and the maintenance of healthy tissues, excess mechanical loading results in traumatic or non-traumatic damage. Thus, to gain intact chondrocytes, it is essential to apply mechanical loading within

* Corresponding authors.
E-mail addresses: mssaidi@sharif.edu (M.S. Saidi), sh_bonakdar@pasteur.ac.ir (S. Bonakdar).

https://doi.org/10.1016/j.jmbbm.2017.12.002
Received 1 October 2017; Received in revised form 29 November 2017; Accepted 1 December 2017
Available online 05 December 2017
1751-6161/

Nomenclature

G	Distance between piston and support [μm]
H	Height of hydrogel [μm]
R	Radius of hydrogel [μm]
r	Radial distance from the centerline of hydrogel [μm]

the physiological range. Limited studies used sole mechanical stimulation for chondrogenic differentiation (Guo et al., 2016; Li et al., 2010; Pelaez et al., 2009; Terraciano et al., 2007; Villanueva et al., 2010), and those which used bioreactors for the stimulation of human-derived cells have been recently reviewed by Khozoee et al. (2016). The strain applied to cells in a three-dimensional culture has a wide range in the literature, from 5% by Neumann et al. (2013) and Neumann et al. (2015) to 40% by Lin et al. (2014). Because of different cell types and sources, various loading protocols (loading per day, frequency, total experiment time, scaffold biomaterial, …) and different culture methods, it is not feasible to compare these studies. To the best of our knowledge, no study has reported the effect of different strains on chondrogenic differentiation.

Numerous benefits of microfluidics have made them a precious tool in biological studies. Microfluidics have enabled automation to facilitate the execution of involved protocols; resulted in folds of decrease in sample and reagent volumes; and had considerable improvement in microenvironment control (Sackmann et al., 2014). One of the emerging fields in which microfluidics have shown their potentials is creating a controlled microenvironment to examine the effects of extrinsic factors on cell fate (Giobbe et al., 2015). Mechanical stimulation in microfluidic platform is noteworthy because of the capability of microfluidics to multiplex different strains on a single platform while minimizing the required cell and reagents.

One of the earliest microfluidics developed for the study of compressive stimuli on cells was introduced by Sim et al. (2007). The authors tried to induce osteogenic differentiation in human mesenchymal stem cells (hMSCs) in a microfluidic compression platform. An arrayed device was used to simultaneously apply five amplitudes of pressure into monolayer cells. Cyclic compression with the frequency of 1 Hz and amplitudes of 1, 5, 10, and 20 kPa were applied to cells. Because of the geometry and method of cell loading, their device was not appropriate to study chondrogenesis, where flattened morphology of cells in mono-layer culture cannot resemble the articular chondrocytes (Panadero et al., 2016). Hosmane et al. (2011) simulated focal compressive injury on single axons using a microfabricated valve-based compression platform. They designed a three-layer microfluidic device that used CO_2 gas to compress the cells with an array of "injury pads" on a poly(dimethylsiloxane) (PDMS) membrane. Cells experienced various pressures, ranging from 0 to 250 kPa, to simulate mild, moderate, and severe levels of axonal injury. Moraes et al. (2010) introduced a microfluidic device to apply three-dimensional compression to encapsulated cells. In this study, the authors fabricated a multilayer PDMS device containing an array of loading pistons. A mixture of hydrogel precursor solution and cells were injected into the device. Transmission of light through a pattern initiated the polymerization process to make cylindrical hydrogels. Because the peripheral face of each cylinder was unconfined, strain uniformity was far from ideal. Moraes et al. (2011) improved their previous design to reduce non-uniformity of strain distribution through the hydrogel using a semi-confined design. Although they promote strain uniformity to a limited extent, nearly half of the hydrogel volume experienced diverse strains which makes their design inappropriate for cellular assays. The most uniform strain distribution is achieved in a fully confined compression microfluidic device, however, fabrication of such a platform is impractical because of limitations in microfabrication and the incapability of the thin PDMS layer to withstand the sticking force between loading piston and chamber (Moraes et al., 2011) More recently, Marsano et al. (2016) proposed a semi-confined compression micro-device to mimic the *in-vivo* microenvironment of native myocardium. Their heart-on-a-chip device recapitulated 10–15% strain in the favorable axis and about −35% strain in unfavorable axis which cannot be neglected.

In the present study, a new semi-confined design is presented to apply a wide range of static and dynamic mechanical compressive strains to cell-laden hydrogels. Numerical methods are employed to investigate the uniformity of strain distribution in cylindrical hydrogels, while convection-diffusion equations are used to ensure the efficacy of mechanical design in providing a suitable microenvironment for cell culture. For the first time, experimentally derived equations by Cochran et al. (2006) for the glycolysis and respiration of cells are integrated into numerical simulation to consider the elaborate nature of cells. Functionality of the device is investigated experimentally by performing 5–20% dynamic compression to induce chondrogenesis. Moreover, viability of cells and gene expression of different mechanical loadings are compared.

2. Numerical methods

2.1. Design of microbioreactor

The new device presented here is a geometrical variation of the semi-confined compression platform of Moraes et al. (2011) which led to substantial improvement in compression uniformity. The device comprises a 5 × 4 array of actuation cavities which enables simultaneous various compressive strains with a single pressurized air source. The schematic cross-sectional view of one of the actuation cavities can be seen in Fig. 1-a. Two PDMS layers consisting of fluidic microchannel networks are separated by a flexible PDMS membrane in the middle. The bottom layer is utilized to push up the loading pistons of the middle layer, and thus pressurized air flows in and is sucked out of this fluidic layer. As the air pressure increases in the microchannels of the bottom layer, the middle membrane experiences an elastic deformation and moves upwards. Subsequently as the air pressure in the microchannels decreases to atmospheric pressure, the middle membrane recovers completely to its original position. Therefore, encapsulated cells in

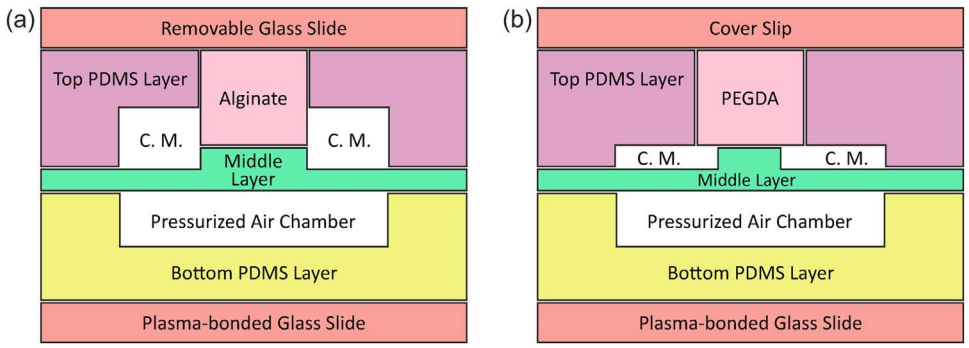

Fig. 1. Schematic cross-section of one of the actuation cavities of the compression platform, (a) Design of current microbioreactor. Piston's diameter is identical to cell-laden alginate hydrogel and thus, as the pressure in air chamber increases, the middle layer moves upwards and uniform axial strain is achieved in the same direction. Each hydrogel is also in contact with culture medium (C. M.) from its peripheral face. (b) Design of Moraes et al. (2011). Piston's diameter is smaller than hydrogel so strain distribution within the PEG hydrogel is not uniform. Culture medium can feed cells in cylindrical hydrogels from their bottom face.