Regenerative Therapy 12 (2019) 2–5

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Review

"Microgravity" as a unique and useful stem cell culture environment for cell-based therapy

Takeshi Imura [a], Takashi Otsuka [a], Yumi Kawahara [b], Louis Yuge [a, b, *]

[a] Division of Bio-Environmental Adaptation Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[b] Space Bio-Laboratories Co., Ltd., Hiroshima, Japan

ARTICLE INFO

Article history:
Received 28 November 2018
Received in revised form
19 February 2019
Accepted 4 March 2019

Keywords:
Microgravity
Stem cells
Regenerative therapy

ABSTRACT

Cell-based therapy using mesenchymal stem cells or pluripotent stem cells such as induced pluripotent stem cells has seen dramatic progress in recent years. Part of cell-based therapy are already covered by public medical insurance. Recently, researchers have attempted to improve therapeutic effects toward various diseases by cell transplantation. Culture environment is considered to be one of the most important factors affecting therapeutic effects, in particular factors such as physical stimuli, because cells have the potential to adapt to their surrounding environment. In this review, we provide an overview of the research on the effects of gravity alteration on cell kinetics such as proliferation or differentiation and on potential therapeutic effects, and we also summarize the remarkable possibilities of the use of microgravity culture in cell-based therapy for various diseases.

Contents

1. Introduction

The progress in regenerative medicine has been rapidly and steadily moving forward. Cell-based therapy, using human mesenchymal stem cells (MSCs), is covered by Japanese medical insurance for graft-versus-host disease [1–3], and clinical trials of cell-based therapy using MSCs are ongoing for various diseases such as brain infarction, spinal cord injury, ischemic heart failure, cartilage defect, rheumatoid arthritis, osteoarthritis, and lupus nephritis [4–12]. In addition, the clinical application of induced pluripotent stem cells has been planned to start within a few years [13]. The important factors in the culture process for improving cell function and therapeutic effects are considered to be cell source (cell selection) [14,15] and culture environment such as soluble factors [16,17], scaffolds [18–20], hypoxia [21], electrical stimulation [22], and gravity [23,24].

Due to flexible adaptation by cells to their surrounding environment, physical stimulus is one of the most important environmental factors that can affect cultured cells. Kawahara et al. reported that electrical stimulation accelerated myoblast

Abbreviations: MSCs, mesenchymal stem cells; CNS, central nervous system; ISS, International Space Station; RCCS, rotary cell culture system; cMSCs, cranial bone-derived MSCs.

* Corresponding author. Division of Bio-Environmental Adaptation Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University, Japan. Fax: 81 82 257 5344.
E-mail address: ryuge@hiroshima-u.ac.jp (L. Yuge).

Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2019.03.001

differentiation through increased expression of connexin 43 protein [25]. Other studies have suggested that physical stimuli such as magnetic field [26,27], shear stress [28], or gravity [23,24,29,30] affects not only cell kinetics, such as cell differentiation and proliferation, but also therapeutic effects following cell transplantation. In particular, gravity is one of the key mechanical stimuli because our bodies are influenced by it at all times [31]. Recent research has shown that therapeutic effects are strongly affected by differences in the gravitational environment to which a cell culture is exposed [23,24]; the unique application of simulated microgravity culture to stem cell-based therapy for central nervous system (CNS) diseases has been reported [30]. Here, we provide an overview of how mechanical loading or unloading affects cells, and we also discuss the unique role, realized and potential, of microgravity culture in cell-based therapy with respect to various diseases.

2. How mechanical loading or unloading influences cells

Every living organism is affected by gravitational force. The RICE experiment during the Space Shuttle STS-95 flight revealed complete morphological changes in rice coleoptiles [32]. At the cellular level, gravity is an important factor for the determination of cell features [23,24,29–32]. Mechanical loading has been suggested as an important determining factor for osteogenic differentiation of MSCs [33] or osteoblast proliferation [34]. Myogenic differentiation is also affected by mechanical loading [35]. In addition, other studies have also suggested the importance of mechanical stress for cell differentiation into tissues such as the smooth muscle of human airways [36], human myocardium [37], keratocytes [38], and dentin-like tissue [39].

It is well known that extracellular force transmission employs membrane-spanning integrins, which connect to the cytoskeleton via talin and paxillin linker molecules [40]. These focal adhesion sites serve as signaling hubs for mechanosensitive kinases such as Fyn and focal adhesion kinase [40]. The cells monitor the surrounding mechanical cues from the extracellular matrix and transmit them through focal adhesion connections to initiate signal pathways that cause reorganization of the cytoskeletal structure [41,42], which allows auto-modulation of signal transmission to the nucleus [43,44]. Interestingly, a recent study showed that mechanical stress regulated stem cell differentiation in the adult Drosophila midgut through the stretch-activated ion channel Piezo [45]. Huang et al. cultured rat MSCs under hypergravity or simulated microgravity, and they reported that hypergravity induced differentiation into force-sensitive cells (cardiomyocytes and osteoblasts), while simulating microgravity-induced differentiation into force-insensitive cells (adipocytes) [46]. Meloni et al. conducted an experiment in space and observed changes in the cytoskeletal structure of human monocyte cells during space flight [47]. They showed that exposure of monocyte cells to microgravity affected the distribution of different filaments and reduced the fluorescence intensity of F-actin fibers. We cultured various cell types such as human osteoblasts [48], human MSCs [49], and mouse embryonic stem cells [50] under simulated microgravity and demonstrated that their differentiation was suppressed in this culture environment. Recently, it has been suggested that factors associated with epigenetics, such as chromatin re-modeling and DNA methylation, contribute to the altered gene expression observed during space flight [51]. We have also reported that myoblast differentiation was attenuated under simulated microgravity because of epigenetic regulation [52].

3. Microgravity devices as a new stem cell culture tool

Researchers are normally able to encounter microgravity environments only in space or during free fall [53–57]. Although an understanding of how microgravity affects our anatomical, physiological, and cellular make-up is important for humanity, space experiments using the International Space Station (ISS) involve a significant cost. Therefore, some simulating microgravity devices have been developed [58–60]. Hoson et al. reported the effects on the vegetative growth phases of plants under conditions of simulated weightlessness (microgravity) using a 3D-clinostat [58]. The National Aeronautics and Space Administration (NASA) has developed a 1D-clinostat, the rotating wall vessel (RWV), a commercially available one-axis rotary cell culture system (RCCS) [57,59,60]. We have also developed a multidirectional gravity control device "Gravite®" (Space Bio-Laboratories, Co., Ltd.), which we have used for simulating microgravity and hypergravity culture conditions (Fig. 1) [30,48–50,52,57,61,62]. By the controlled rotation of the two axes, this device minimizes the cumulative gravity vector at the center of the device, generating an average of $10^{-3} \times g$ over time.

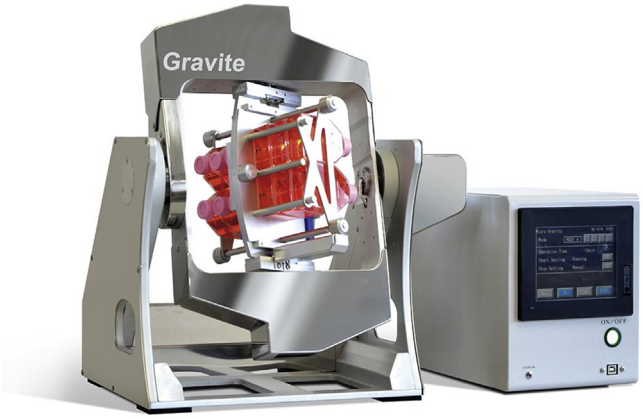

Fig. 1. Multidirectional gravity control device "Gravite®" by the controlled rotation of two axes, this device minimizes the cumulative gravity vector at the center of the device, generating an average of $10^{-3} \times g$ over time. It can also realize 2 g or 3 g environment by the controlled rotation of one axis.

The development of such devices has led not only to achieving a better understanding of the microgravity phenomenon but also of its application to diverse objectives [30]. Recently, microgravity has been recognized as a novel and unique culture environment for stem cells because of the unique cell changes that can occur in microgravity culture.

4. Microgravity culture enhances the therapeutic effect of stem cells transplantation

Due to changing the cell characteristics, microgravity culture has the possibilities of being useful cell culture environment for cell-based therapy. Monticone et al. reported interesting results from their space experiment performed from March 30 to April 8, 2006 (experiment "Stroma-2") [63]. In this experiment, they cultured murine bone marrow stromal cells inside the ISS and compared them with control cells cultured under $1 \times g$ (1G) culture conditions, and they found that most of the differential gene expressions between cells under the two gravity levels were related to neural development, neuron morphogenesis, and transmission of nerve impulse and synapse. Mattei et al. also reported differential gene expression involved in rostral-caudal neural patterning and cortical markers in simulated microgravity culture using RCCS [64]. From those findings, it was demonstrated that a microgravity environment affects cell characteristics, in particular nervous system-related genes, and we hypothesized that cells cultured under microgravity may exhibit altered therapeutic effects if used

for cell-based therapy with respect to CNS diseases. We recently demonstrated the effect of microgravity culture on the neuroprotective effects of cranial bone-derived MSCs (cMSCs) [24]. In this research, we demonstrated greater expression of genes involved in neuroprotective effects (anti-inflammation and anti-apoptosis), such as hepatocyte growth factor and transforming growth factor beta, in cMSCs cultured under microgravity conditions than in those cultured under 1G conditions. In addition, cMSCs cultured under microgravity showed higher neuroprotective effects in vitro and in vivo than the corresponding cells grown under 1G conditions. Moreover, Yuge et al. and Mitsuhara et al. also demonstrated remarkable therapeutic effects of MSCs cultured under simulated microgravity toward spinal cord injury or traumatic brain injury, respectively [23,61]. From our results, microgravity culture conditions appear to have enormous potential as a cell culture environment for developing cultured cells with improved therapeutic effects. Chen et al. also reported that rat MSCs subjected to simulated microgravity using a clinostat secrete more neurotrophins, such as nerve growth factor, brain-derived neurotrophic factor, and ciliary neurotrophic factor [65].

Application of simulated microgravity culture is spreading, not only for cell therapy for neural systems but also for treatment of other diseases. Hagiwara et al. showed an interesting application of microgravity culture in vasculogenesis and tissue regeneration [66]. They demonstrated that initial cultivation under microgravity conditions followed by cultivation under a 1G environment was shown to increase endothelial progenitor cell expansion rates and angiogenic potential to a remarkable degree. Imura et al. also suggested the use of temporal microgravity culture followed by 1G culture [67], a strategy which is one of the key options for microgravity applications to cell culture. In addition, it has been reported that dynamic three-dimensional simulated microgravity culture might contribute to tooth tissue regeneration using human dental pulp stem cells [68]. Moreover, microgravity culture has potential in the field of visceral diseases. Wang et al. described the generation of functional hepatic-like cells from mouse embryonic stem cells using a biodegradable polymer scaffold and a simulated microgravity culture environment, wherein the cells generated showed transplantable capacity (in terms of the differentiation and maturation of hepatic-like cells) in vivo [69]. In the field of diabetes, tissue-like aggregates composed of Sertoli cells and islets cultured in microgravity environment were transplanted into rats with type I diabetes and subsequently showed increased tolerance to glucose [70]. These findings demonstrate that cell culture under microgravity shows exciting potential for application to cell-based therapy of various diseases.

Declarations of interest

LY is the director of Space Bio-laboratories Co., Ltd (SBL), and YK is the president of SBL. They share holding. The conflicts of interest for this research have been approved by the Conflict of Interest Management Committee. By regularly reporting the research progress to the Conflicts of Interest Management Committee, we will maintain fairness regarding the interests of this research.

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