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

A R T I C L E   I N F O

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.

© 2019, The Japanese Society for Regenerative Medicine. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Contents

1. Introduction ................................................................. 2
2. How mechanical loading or unloading influences cells .................... 3
3. Microgravity devices as a new stem cell culture tool ...................... 3
4. Microgravity culture enhances the therapeutic effect of stem cells transplantation ................................................................. 3
Declaration of interest ................................................................ 4
References .............................................................................. 4

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
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. It can also realize 2 g or 3 g environment by the controlled rotation of one axis.

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 Mitsuhashi 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 nervous 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.

References

[1] Muroi K, Miyamura K, Okada M, Yamashita T, Murata M, Ishikawa T et al. Bone marrow-derived mesenchymal stem cells (BM-MSC) for acute myocardial infarction: a phase II study. Jpn J Pharmacol 2010;103:243–50.
[2] Le Blanc K, Rasmusson I, Sundberg B, Gorthérström C, Hassain M, Uzunel M et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 2004;363:1439–41.
[3] Le Blanc K, Frassoni F, Ball I, Locatelli F, Roelofs H, Lewis I et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft–versus-host disease in a phase II study. Lancet 2008;371:570–7.
[4] Shichinohe H, Kawabori M, Iijima H, Teramoto T, Abumiya T, Nakayama N et al. Research on advanced intervention using novel bone marrow (BM) stem cells (RAINBOW): a study protocol for a phase I, open-label, uncontrolled, doseescalation trial of autologous bone marrow stromal cell transplantation in patients with acute ischemic stroke. BMC Neurol 2017;17:17.
[5] Honmou O, Houlon K, Matsuura T, Nitsu Y, Ishiai S, Onderka R et al. Intravenous administration of autologous stem expanded autologous mesenchymal stem cells in stroke. Stroke 2011;42:1790–96.
[6] Oh SK, Choi KH, Yoo JY, Kim DY, Kim SJ, Jeon SR. A phase III clinical trial showing limited efficacy of autologous mesenchymal stem cell therapy for spinal cord injury. Neurosurgery 2016;78:436–47.
[7] Hof JW, Cho TH, Park DH, Lee JB, Park YJ, Chung YG. Intrathecal transplantation of autologous adipose-derived mesenchymal stem cells for treating spinal cord injury: a human trial. J Spinal Cord Med 2013;36:595–65.
[8] Perin EC, Borow KM, Silva GV, DeMaria AN, Marroquin OC, Huang PP et al. A phase II dose-escalation study of allogeneic mesenchymal precursor cells in patients with ischemic or nonischemic heart failure. Circ Res 2015;117:576–84.
[9] Wong KL, Lee KB, Tai BC, Law P, Lee EH, Hui HJ. Injectable cultured bone marrow-derived mesenchymal stem cells in varus knee with cartilage defects undergoing high tibial ostotomy: a prospective, randomized controlled clinical trial with 2 years’ follow-up. Arthroscopy 2013;29:2020–8.
[10] Park EH, Lim HS, Lee S, Roh K, See KW, Kang KS et al. Intravenous infusion of umbilical cord blood derived mesenchymal stem cells in hemolytic arthrits: a phase Ia clinical trial. Stem Cells Transl Med 2018;7:636–42.
[11] Al-Najjar M, Khalil H, Al-Ajoum An, Antlary E, Hamdan M, Rahman R et al. Intravenous injection of ex vivo expanded autologous bone marrow mesenchymal cells in moderate and severe knee osteoarthritis is safe: a phase II pilot study. J Orthop Surg Res 2017;12:190.
[12] Deng D, Zhang P, Guo Y, Lin TM. A randomized double-blind, placebo-controlled trial of allogeneic umbilical cord-derived mesenchymal stem cell for lupus nephritis. Ann Rheum Dis 2017;76:1436–9.
[13] Nishimura K, Takashashi J. Therapeutic application of stem cell technology toward the treatment of Parkinson’s disease. Biol Pharm Bull 2013;36:171–3.
[14] Aibiki M, Mitsuhashi T, Okazaki T, Imura T, Nakagawa K, Otsuka T et al. Rat cranial bone-derived mesenchymal stem cell transplantation promotes functional recovery in ischemic stroke model rats. Stem Cell Dev 2018;27:1053–61.
[15] Robl JM, Mori M, Cerullo D, Introna M, Calpani O, Corna D et al. Therapeutic potential of stromal cells of non-renal or renal origin in experimental chronic kidney disease. Stem Cell Res Ther 2018;9:220.
[16] Lin T, Pajarinen J, Nabheshima A, La L, Nathan K, Jansen EI et al. Preconditioning of murine mesenchymal stem cells synergistically enhanced immunomodulation and osteogenesis. Stem Cell Res Ther 2017;8:277.
[17] Yao Y, Zhang F, Wang L, Zhang C, Wang Z, Chen J et al. Lipopolysaccharide preconditioning enhances the efficacy of mesenchymal stem cells transplantation in a rat model of acute myocardial infarction. J Biomed Sci 2009;16:74.
[18] Tajima S, Tabata Y. Preparation of epithelial cell aggregates incorporating matrigel micropherses to enhance proliferation and differentiation of epithelial cells. Regen Ther 2017;7:34–44.
[19] Inoo K, Bando H, Tabata Y. Enhanced survival and insulin secretion of insulinoma cell aggregates by incorporating gelatin hydrogel microspheres. Regen Ther 2018;8:29–37.
[20] Shi W, Huang CJ, Xu XD, Jin GH, Huang RQ, Huang JF et al. Transplantation of RADA16-BDNF peptide scaffold with human umbilical cord mesenchymal stem cells forced with CXCR4 and activated astrocytes for repair of traumatic brain injury. Acta Biomater 2016;45:247–61.
[21] Imura T, Tomiyasu M, Otsuru N, Nakagawa K, Otsuka T, Takahashi S et al. Hypoxic preconditioning increases the neuroprotective effects of mesenchymal stem cells in a rat model of spinal cord injury. J Stem Cell Res Ther 2017;7:375.
[22] Matsunoto M, Imura T, Fukazawa T, Sun YN, Takeda M, Kajiume T et al. Electrical stimulation enhances neurogenin2 expression through β-catenin signaling pathway of mouse bone marrow stromal cells and intensifies the effects of cell transplantation on brain injury. Neurosurg Lett 2013;53:71–6.
[23] Yuge L, Sasaki A, Kawahara Y, Wu SL, Matsunoto M, Manabe T et al. Simulated microgravity maintains the undifferentiated state and enhances the neural repair potential of bone marrow stromal cells. Stem Cell Dev 2011;20:900–9.
[24] Otsuka T, Imura T, Nakagawa K, Shrestha L, Takahashi S, Kawahara Y et al. Simulated microgravity culture enhances the neuroprotective effects of human cranial bone-derived mesenchymal stem cells in traumatic brain injury. Stem Cell Dev 2018;27:1287–97.
[25] Kawahara Y, Yamaoka K, Iwata M, Fujimura M, Kajiume T, Magaki T et al. Novel electrical stimulation sets the cultured myoblast contractile function to ion. Pathobiology 2006;73:238–51.
[26] Yuge L, Katoaka K. Differentiation of myoblasts is accelerated in culture in a magnetic field. In Vitro Cell Dev Biol Anim 2000;36:383–6.
[27] Yuge L, Okubo A, Miyashita T, Kumagai T, Nakata T, Takada S et al. Physical stress by magnetic force accelerates differentiation of human osteoblasts. Biochem Biophys Res Commun 2003;311:32–8.
Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of
Sen B, Xie Z, Case N, Thompson WR, Uzer G, Styner M, et al. mTORC2 regulates
Hashmi B, Mammoto T, Weaver J, Ferrante T, Jiang A, Jiang E, et al. Mechanical
Ferrell N, Cheng J, Miao S, Roy S, Fissell WH. Orbital shear stress regulates
Ruan JL, Tulloch NL, Saiget M, Paige SL, Razumova MV, Regnier M, et al. Me-
Asano S, Ito S, Morosawa M, Furuya K, Naruse K, Sokabe M, et al. Cyclic stretch
Kapur S, Mohan S, Baylink DJ, Lau KH. Fluid shear stress synergizes with
Ozcivici E, Luu YK, Adler B, Qin YX, Rubin J, Judex S, et al. Mechanical signals as
Yuge L, Hide I, Kumagai T, Kumei Y, Takeda S, Kanno M, et al. Cell differen-
Hoson T, Soga K, Mori R, Saiki M, Wakabayashi K, Kamisaka S, et al. Stem cell culture in microgravity and its application in cell-based therapy. Stem Cell Dev 2018;27:1298–302.
Herranz R, Medina FJ. Cell proliferation and plant development under novel altered gravity environments. Plant Biol 2014;16:23–30.
Hoson T, Soga K, Mori R, Saiki M, Wakabayashi K, Kamisaka S, et al. Morphogenesis of rice and Arabidopsis seedlings in space. J Plant Res 1999;112:477–86.
Ozcivici E, Luu YK, Adler B, Qin YX, Rubin J, Judex S, et al. Mechanical signals as analobic agents in bone. Nat Rev Rheumatol 2010;6:50–9.
Kapur S, Mohan S, Baylink DJ, Lau KH. Fluid shear stress synergizes with insulin-like growth factor-1 (IGF-I) on osteoblast proliferation through integrin-dependent activation of IGF-I mitogenic signaling pathway. J Biol Chem 2000;275:20163–70.
Ciofani G, Ricotti L, Rigosa J, Menciassi A, Mattoli V, Monici M. Hydrostatic effects on myoblast proliferation and differentiation. J Biosci Bioeng 2012;113:258–61.
Asano S, Ito S, Morosawa M, Furuya K, Naruse K, Sokabe M, et al. Cyclic stretch enhances reorientation and differentiation of 3-D culture model of human airway smooth muscle. Biochem Biophys Res 2018;16:32–8.
Ruan J, Tulloch NL, Saiget M, Paige SL, Razumova MV, Regnier M, et al. Mechanical stress promotes maturation of human myocardium from pluripotent stem cell-derived progenitors. Stem Cell Protoc 2015;33:2148–57.
Ferrell N, Cheng J, Miao S, Roy S, Fissell WH. Orbital shear stress regulates differentiation and barrier function of primary renal tubular epithelial cells. Am Soc Artif Intern Organs J 2018;64:74–67.
Hashmi B, Mammoto T, Weaver J, Ferrante T, Jiang A, Jiang E, et al. Mechanical induction of dentin-like differentiation by adult mouse bone marrow stromal cells using compressive scaffolds. Stem Cell Res 2017;24:55–60.
Uzer G, Fuchs RK, Rubin J, Thompson WR. Plasma and nuclear membranes convey mechanical information to regulate mesenchymal stem cell lineage. Stem Cell 2016;34:1455–63.
Seo B, Xie Z, Case N, Thompson WR, Uzer G, Stynner M, et al. INTROR2 regulates mechanically induced cytoskeletal reorganization and lineage selection in marrow-derived mesenchymal stem cells. J Bone Miner Res 2014;29:79–88.
Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science 2005;310:1139–41.
Hu S, Chen J, Butler JP, Wang N. Prestress mediates force propagation into the nucleus. Biochem Biophys Res Commun 2005;329:423–8.
Uzer G, Thompson WR, Sen B, Xie Z, Yen SS, Miller S, et al. Cell mechanosensitivity to extremely low-magnitude signals is enabled by a LiNcd nucleus. Stem Cell 2015;33:2063–76.
He L, Gi S, Huang J, Samuel ADT, Perrimon N. Mechanical regulation of stem cell differentiation by the stretch-activated Piezo channel. Nature 2018;555:103–6.
Huang Y, Dai QZ, Ling SK, Zhang HY, Wan YM, Li YH. Gravity, a regulation factor in the differentiation of rat bone marrow mesenchymal stem cells. J Biomed Sci 2009;16:87.
Meloan MA, Gallièri C, Pani G, Saba A, Pippa P, Cogoli-Greuter M. Space flight affects motility and cytoskeletal structures in human monocyte cell line J-111. Cytoskeleton 2011;68:125–37.
Yuge E, Hide I, Kumagai T, Kumei Y, Takeda S, Kanno M, et al. Cell differentiation and p38 (MAPK) cascade are inhibited in human osteoblasts cultured in a three-dimensional climostat. In Vitro Cell Dev Biol Anim 2003;39:89–97.
Yuge E, Kajiume T, Tahara H, Kawahara Y, Umeda C, Yoshimoto R, et al. Microgravity potentiates stem cell proliferation while sustaining the capability of differentiation. Stem Cell Dev 2006;15:921–9.
Kawahara Y, Manabe T, Matsumoto M, Kajiume T, Matsumoto M, Yuge E. Life free embryonic stem cell culture in simulated microgravity. PLoS One 2009;4:e6343.