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Microgravity may help future organ/tissue manufacture

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Stem cells are a group of multipotent or pluripotent cells which could differentiate into specialized cells or could self-renew to duplicate themselves. During the body developing period, stem cells differentiated into specialized cells to build the whole body. In the adult organisms, stem cells live in seclusion and are ready to repair the injuries of tissues. Within one tissue, stem cells could differentiate in to all types of somatic cells of the tissue, for example, within central nervous system (CNS), neural stem cells could differentiated in to neurons, astroglias, and oligodendrocytes, which take together to build the CNS. When researches trying to construct a tissue or an organ *in vitro*, the first question to be solved is how to induce the differentiation of stem cell as we supposed. Thus, stem cells are considered as the key factor of tissue engineering aiming organ/tissue manufacture.

Microgravity is condition of weightlessness, where the G-force is nearly to zero. It is a physical force which could affect functions of cell and even the whole body. Recently, researchers paid attention to stem cells under microgravity environments. Distinguishing from the physics or chemistry experiments under microgravity, it would take much more time to detect the impacts of microgravity on stem cells. The microgravity approaches were realized by simulations on the earth or by space flights. On the earth, the devices which could generate multidirectional G force, such as three-dimensional (3D)-clinostat, horizontal cyclotron bioreactor, 3D gyroscope and rotary cell culture system (RCCS), produced an environment with an average G of 10^{-3}. However, such devices could only provide the average microgravity. The long-period microgravity experiment could only perform in space facilities, such as space station, space shuttle and satellite. There are experiments of neural stem cells (NSCs), hematopoietic stem cells (HSCs) and bone mesenchymal stem cells (BMSCs) loaded onto the SJ-10 satellite which was launched at Apr 6, 2016 (Hu et al., 2014). Researchers have developed novel devices which control the medium exchange precisely to provide environments for the proliferation and differentiation of stem cells. After the 12-day space flight, cell samples were harvested and mechanisms of self-renewal and differentiation of stem cells would be profoundly evaluated.

One direct effect of the loss of gravity force on cells was the changes of cytoskeleton and adhesion. Cytoskeleton and adhesion were involved in cell morphology, intracellular transportation, and intercellular communication. These processes contributed greatly to self-renewal and differentiation of stem cells. Different types of stem cells responded to the stimulation of microgravity (by either spaceflight or ground-simulation) variously. Microgravity enhanced the efficiency of hepatocyte differentiation of mESCs (Wang et al., 2012), and it also supported the pluripotency of ESCs at the absent of LIF or serum and feeder layer, moreover, the proliferation of the undifferentiated ESCs was greatly raise by ~8 times compared with that of cells in the 1G environment (Kawahara et al., 2009). Researches on the relation-

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ship between mesenchymal stem cells (MSCs) and gravity were more intensive because of the multipotency of MSCs. The differentiation of MSCs was sensitive to gravity. Hypergravity induced MSCs to differentiate into force-sensitive cells such as osteoblasts and myocardial cells, however, microgravity may guide MSCs to force-insensitive cells such as adipocytes (Huang et al., 2009). As to HSCs, the migration of bone marrow $CD34^+$ cells was significantly reduced under microgravity (Plett et al., 2004). The cycle was arrested at G0/G1 phase, thus the proliferation of hematopoietic precursors was inhibited by microgravity (Domaratskaya et al., 2002). In addition, microgravity was found promoting the proliferation of periodontal ligament stem cells (PDLSCs) and liver stem cells (Li et al., 2009). The viability of NSCs and epidermal stem cells (EpSCs) were found to be enhanced by RCCS-simulated microgravity (Chiang et al., 2012; Lei et al., 2011).

As one of the key factors of tissue/organ manufacture, there are two behaviors of stem cells deserved special attentions: one is proliferation, expansion in large scale keeping undifferentiated state; the other is differentiation, development into desired specific cell types. These are the two important questions to be solved in tissue/organ manufacture studies. 3D culture was found to promote the stemness or self-renewal states of stem cells, which indicated that spatial or/and mechanical factors may act on self-renewal of stem cells. Cell shapes were reported to involve in the cell fate decision. Inhibition of cell spreading promoted the pluripotency of mESCs at the absent of LIF (Murray et al., 2013). Coincidently, microgravity could also support the expansion of mESCs without the need of LIF (Kawahara et al., 2009). Cytoskeletons and adhesion molecules connected tightly to cell shapes and felt intracellular mechanical signals. These results suggested that spatial or mechanical factors could regulate the self-renewal of stem cells by effecting cytoskeleton and adhesion. Microgravity was found to disrupt the distribution of adhesion molecules and cytoskeleton, Re-organization of cytoskeleton may result in the preservation of undifferentiated statement of stem cells (Figure 1). From this, the microgravity may be a stem cell expansion factory to produce the seed cells for tissue engineer. On the earth, the multipotency of NSCs was hard to be maintained during long-term scalable expansion by traditional culture system. Considering that the stemness of NSCs and MSCs were promoted under 3D culture system, we are wondering what impacts would be made by microgravity accompanied by 3D culture towards stem cells. On the SJ-10 satellite, for the first time, 3D-cultured NSCs proliferated and differentiated for 12 days under space microgravity environments. The proliferation efficiency of NSCs would be evaluated. The other behavior of stem cells important for organ manufacture is directed differentiation. ES cells could differentiate into any kind of cells, whereas adult stem cells of special tissue could differentiate into several cell types within special tissue. However, how to induce one kind of stem cells totally differentiate into one cell type or differentiate into one type by large percentage remained a challenge for tissue engineering researchers. We are interested that the mechanical factor of gravity could lead the differentiation of stem cells. Studies on MSC differentiation have shown that different gravity forces lead MSCs to differentiate into different cell types: hypergravity to osteoblasts and myocardial

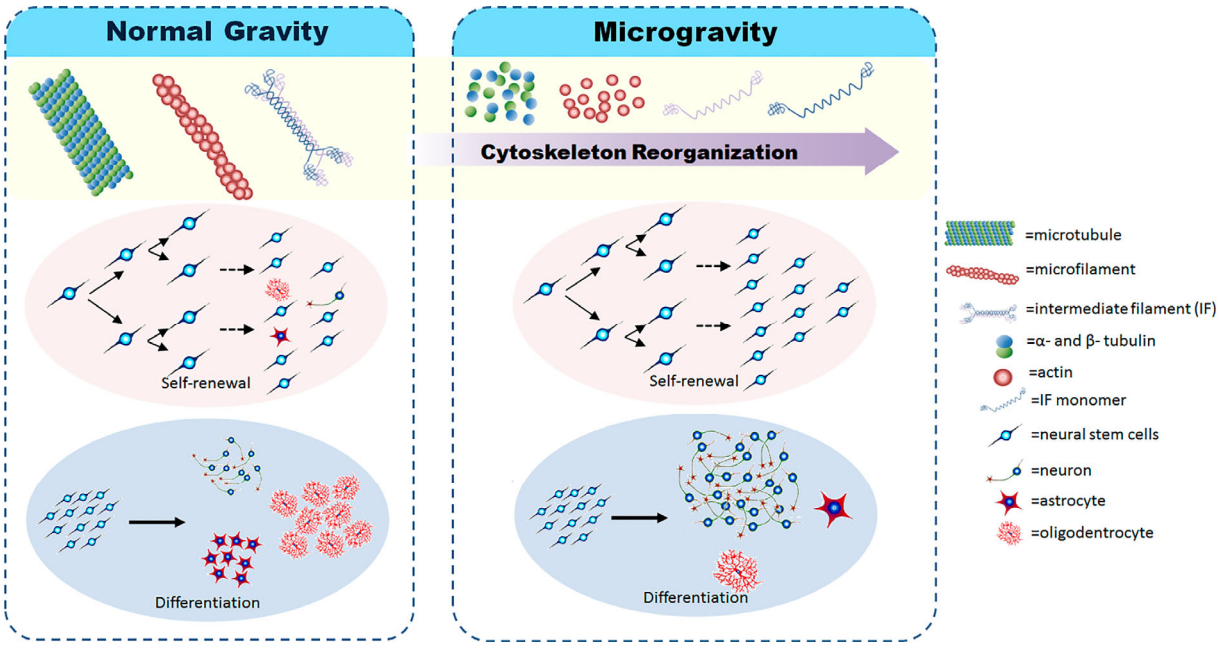

Figure 1 Diagram taking neural stem cells for an instance to illustrate the self-renewal and differentiation regulated by microgravity.

cells which are sensitive to force; microgravity to adipocytes which are insensitive to force. As to NSCs, our knowledge about the relationship between neural differentiation and gravity is still poor. Researchers have found that the rigidity of extracellular matrix (ECM) was an influential factor towards neural differentiation. On deformable substrates, compliant matrix, other to stiffer substrates, MSCs tended to neuronal phenotypes (Engler et al., 2006). The growth of neuron was promoted on compliant matrix over that of glia cells (Georges et al., 2006). Moreover, the matrix stiffness was found to regulate neuronal axon development. Gravity could be seen as a kind of mechanics effects. At the same time, it is interesting that the marker genes of neuron mainly were cytoskeleton protein or protein associated to cytoskeleton. For example, immature neuron marker TUJ1 (β-tubulin III) was a kind of microtubule; NSCs marker nestin and glia cell marker GFAP (glial fibrillary acidic protein) both belong to interfilaments; mature neuron marker MAP2(microtubule-associated protein 2) and immature neuron marker DCX (Doublecortin) both are microtubule-associated proteins. We speculated the microgravity may affect the neural differentiation even preferentially induce one neural cell type (Figure 1). The ECM mechanics has shown that neurons were more adaptive to weak force environments. So it is probable that microgravity tend to induce neuronal differentiation. Neuronal differentiation from NSCs or neuron production was one barrier for nervous tissue regeneration. At one hand, once the neuron of central nervous system was injured, it could not be recovered by itself. At the other hand, it is difficult to induce one subtype of neuron greatly differentiated from NSCs, for glia differentiation could not be blocked. If microgravity may tendentiously guide neuronal differentiation, the space would be a novel suitable environment for neuron production. NSCs sample loaded on SJ-10 satellite have been recovered after 12-day spaceflight. Our hypothesis will be verified by a series of biological experiments.

From above, it is likely that microgravity may enhance self-renewal and differentiation of stem cells. For the future tissue/organ manufacture, microgravity could do more: on one side, the stemness of stem cells could be preserved in large scaled/passaged expansion; on the other side, the direction of differentiation of stem cells under microgravity may be different from that under normal gravity. Although the efficiency of microgravity acting on self-renewal and differentiation of stem cells diverse from cell types, there is no doubt that microgravity is a novel opportunity for stem cell and tissue/organ manufacture researches. Bioreactors based on microgravity would produce large amount of stem cells or specific type of cells, which may provide a novel cell sources of tissue/organ manufacture. With the development of the space technology of China, Shenzhou craft, Tiangong 2 and 3 will be launched to build our own space station. At that time, the space cell culture technology and conditions would be much more advanced. Microgravity provided by spaceflight was much more stable than that simulated on ground. Scalable cells for future tissue/organ manufacture could be fabricated in bioreactors in space station. Furthermore, along with the 3D cell culture and 3D bioprinting technology progressing, stem cells or mature cells would be assembled by automatic instruments. Tissue/organ manufacture could be attempted at the space station. We are looking forward to the development of space biofabrication technology to present opportunities for tissue/organ manufacture.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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