Trends in Biotechnology

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Magnetic levitation for space exploration

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Magnetic levitation allows for simulating the microgravity conditions to advance bottom-up tissue engineering, forging regenerative medicine ahead to enable space exploration. Here, magnetic levitation methods for microgravity studies and the biofabrication of 3D cellular structures are discussed.

Tissue engineering in space

Space exploration expeditions such as the journey to Mars, which is 363 million km from Earth, require long-duration space flights. One of the main prerequisites for these flights is self-sufficiency instead of relying on cargo from Earth. Understanding the effects of space's microgravity or different gravity levels of other planets on the human body in the long term is a fundamental necessity for space expeditions. Space agencies are striving to expand the frontiers of knowledge, capability, and opportunity in space by seeking methodologies to engineer artificial components to support human life and to enable the colonization of space. The development and long-term deployment of living engineered filters can be a game-changer in resource-limited settings; for example, limited carbon (organic matter), minerals, and nutrients. Additionally, creating tissue constructs and organoids in space not only advances space regenerative medicine and medical self-sufficiency for astronauts but also offers a platform to evaluate the effects of gravity levels on 3D tissue structures [1]. 3D bioprinting technologies are promising tools for creating *in vitro* 3D models to mimic tissue and organ constructs. The integration of 3D bioprinting with microgravity can advance the frontiers of space medicine. Emulating the effects of microgravity through magnetic levitation can enable studying the experimental conditions for prognosticating their outcome and troubleshooting the conceivable issues.

Magnetic levitation for engineered microgravity settings

Magnetic levitation through negative magnetophoresis (diamagnetophoresis) has been used to simulate the weightlessness condition. Benefiting from this simulated environment, the biofabrication of 3D cellular structures can be facilitated. For instance, a 3D cell culture with self-assembly ability was biofabricated *in situ*. The microgravity-mimicking environment was created with magnetic levitation using gadolinium (Gd) solutions [2]. A microfluidic device was developed to levitate solutions containing bone marrow mesenchymal stem cells (MSCs) with different concentrations of Gd. By considering cell viability and levitation position in the setup, optimal chelate form and Gd concentration for levitation and cultivation of the cells were determined. *In situ* assemblies in both short- and long-term levitations were also studied with different cell numbers. Furthermore, a breast cancer cell line was cultured with bone marrow MSCs to biofabricate different biphasic cellular forms in the levitation system. This experiment allowed for investigation of the self-assembly process with various cell–cell adhesion characteristics during weightlessness, resulting in looser cell clusters as compared to that of bone marrow MSCs. 3D cell cultures in a magnetic levitation setup enabled the evaluation of microgravity effects on cells and molecules with real time imaging. One limitation of 3D spheroid formation using magnetic levitation is the diversity in the particular levitation level to which cells are mustered, depending on the cell types and their density [2]. This can be a more challenging obstacle for clusters having more than one type of cell. Although the most suitable chemical composition and concentration of Gd ions was chosen based on the viability of cells, the cellular effects of various concentration of Gd ions, especially in long-term culture, need to be studied. Paramagnetic solutions based on Gd for magnetic resonance imaging in humans have been shown to be non-toxic, iso-osmolar in concentrations required for imaging, and completely compatible with human blood cells [3]. Furthermore, the same study has investigated the potential harmful effects of the Gd solutions (5–40 mmol/l) on cell viability in periods of 36 h, demonstrating that viability of the samples treated with gadolinium (17.3–19.5%) was comparable with the buffer-treated samples (19.1%) [3].

Magnetic levitation can also be used for the fabrication of living soft constructs [4]. Microstructures such as cell encapsulating hydrogels or cell-seeded microbeads were aligned in Gd-containing media featuring paramagnetic properties for 2/3D assembly with contactless controlling. These microstructural units were fabricated in two ways: (i) using polymers such as methacrylated gelatin (GelMA) or poly(ethylene glycol) dimethacrylate (PEGDA) through photolithography, followed by UV-initiated crosslinking; and (ii) cell-seeded microbeads fabricated by laminin coating followed by incubation in cell suspension. Two neodymium magnets (NdFeB) with same poles facing each other created a microgravity environment (Figure 1A). The particles in the medium move from a high magnetic field strength to the weaker if their magnetic susceptibility is lower than magnetic susceptibility of the suspending medium. At equilibrium point, a combination of magnetic (F_m) and corrected gravitational (F_g) forces acts on the levitating particles. By altering the distance between the magnets and changing the concentration of the solution, precise controlling and levitation of small particles were achieved (Figure 1B). This

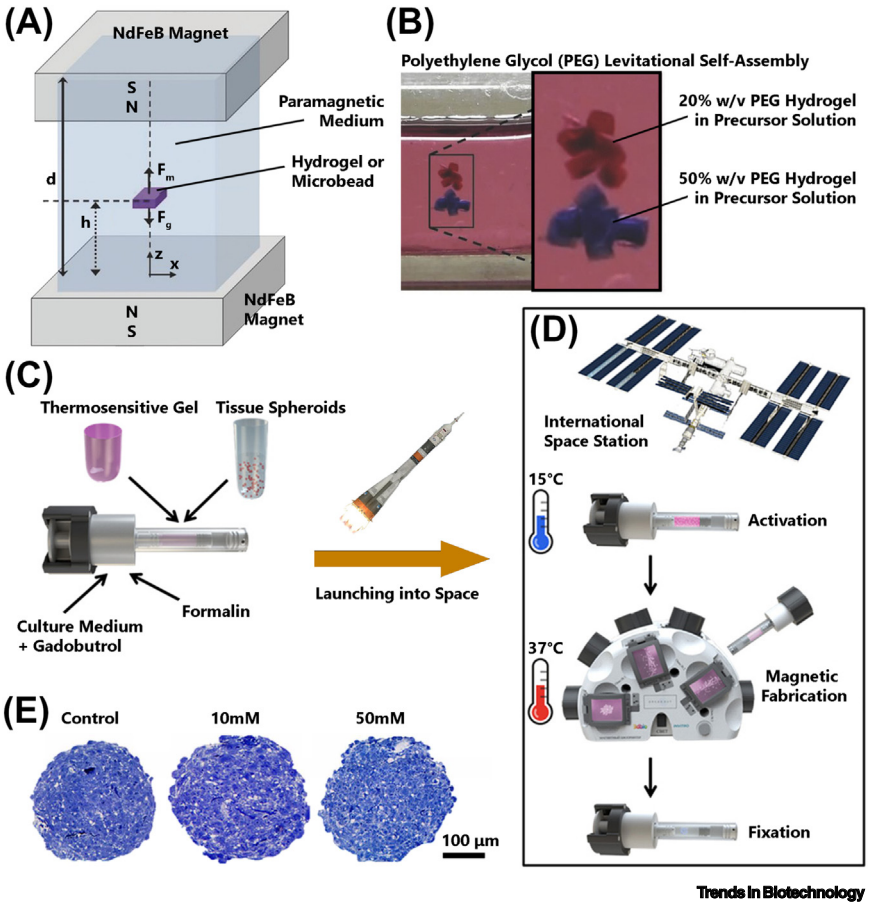

Figure 1. Magnetic levitation in microgravity studies. (A) Soft living material fabrication using a magnetic levitation platform with two neodymium magnets. (B) Selective levitational assembly of PEG hydrogel samples. A and B are reproduced, with permission, from [4]. (C) A magnetic levitational bio assembly setup was developed for the fabrication of 3D tissue constructs in the International Space Station (ISS). First, the cuvettes were filled with chondrospheres in thermoreversible nonadhesive hydrogel, culture medium with paramagnetic gadobutrol, and formalin. (D) Second, the cuvettes were transferred to ISS and activated by decreasing their temperature to 15°C. The magnetic levitational bioassembly of 3D tissue constructs were performed at 37°C, and then they were stabilized. (E) Chondrospheres without or after 24 h exposure to gadobutrol (10 mM and 50 mM); toluidine blue staining. C–E were reproduced, with permission, from [5] in accordance with the Creative Commons Attribution (CC BY) license.

study presented a fabrication method for soft living constructs in weightlessness conditions for application in bottom-up tissue engineering.

Bioassembly of 3D structures can also be carried out via magnetic levitation in microgravity studies for the fabricated 3D tissue structures. A customized setup has been developed for the fabrication of 3D cartilage tissue constructs in the International Space Station (ISS) [5]. Before transportation to space, the cuvettes were filled with tissue spheroids (chondrospheres) in thermoreversible nonadhesive hydrogel for viable protection of tissue spheroids to prevent the unwanted initial fusion or spreading of the tissues (Figure 1C). The culture medium of the solution consisted of different concentrations of biocompatible paramagnetic Gd^{3+}-chelate gadobutrol, and formalin was used as the fixative solution. Upon the completion of the preparation process, the cuvettes were launched to the ISS (Figure 1D). Decreasing the temperature to 15°C activated the cuvettes. The magnetic levitational bioassembly of the 3D cartilage tissue constructs was performed at 37°C, followed by stabilization. The cuvettes were then sent back to Earth for testing. The chondrospheres' viability was analyzed with a quantitative assessment after 72 h incubation in Mebiol gel and culture medium. The developed tissue structures exhibited an appropriate level of viability (97±6%) along with progressive steps of the tissue spheroid fusion procedure. Gadobutrol, the leading constituent of the paramagnetic solution for magnetic levitation setup, was another factor that contributed to the viability and physiology of chondrospheres. To study this relationship, chondrospheres were exposed to gadobutrol (10 and 50 mmol/l) (Figure 1E). No significant apoptosis and cell death in the spheroids was observed at low gadobutrol concentration; however, some softening and reduction of roundness in chondrospheres was detected in high gadobutrol concentrations. This study demonstrated the contribution of magnetic levitation for the bioassembly of 3D tissue structures from tissue spheroids in microgravity.

Concluding remarks

Magnetic levitation systems offer advantages of being cost- and time-efficient in comparison to other 3D cell culture methods such as polymer scaffolds and protein gel substrates [6]. This approach does not require the fabrication of scaffolds and other sacrificing structures and does not necessitate particular serum media in the process [6]. Magnetic levitation can influence the viability of biological samples especially in long-term experiments. The paramagnetic medium should be selected based on the application and samples to be levitated [7]. Additionally, the concentration of the paramagnetic solution should be optimized for the setup under study, as a higher concentration can alter cell densities [7]. Osteogenesis

differentiation in the extracellular environment of 3D spheroids culture systems has been studied under magnetic levitation for bone tissue engineering applications [8]. A troubleshooting protocol has been provided to address the potential challenges in 3D cell culturing by magnetic levitation [6].

Magnetic levitation platforms are versatile tools that either on Earth or in space can be used for studying gravity effects in biomedical research. They also enable applications in bottom-up biofabrication of tissues and organs with more complex 3D designs with scaffold-free structures [7]. The integration of magnetic levitation with 3D printing and 3D bioprinting technologies can allow for facile manufacturing with scarce materials to a limited extent in space [9–11]. 3D printing is a scalable method that can be deployed beyond the ISS. Designing microgravity laboratories that orbit the Earth can be an alternative approach. Major opportunities of biomanufacturing in low Earth orbit (LEO), enabled by the role of microgravity, taking its impact and risk into account, include disease modeling such as muscle wasting (weakening, shrinking, and/or loss of muscles because of a disease or lack of use), heart diseases, osteoarthritis, aging, and biofouling), and stem-cell-derived products with enhanced functionalities [12]. The Biomanufacturing in Space Symposium accentuating biomanufacturing opportunities in LEO along with the exploration of commercial opportunities and market analysis were recently reported [12]. Additionally, Artificial Intelligence (AI) techniques such as machine learning and deep learning can be helpful in eliminating the need to send an operator to space by delegating the experimental responsibilities to the AI core, providing faster analysis, and forecasting the data [13].

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Declaration of interests

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