Chapter

Magnetic Levitation Based Applications in Bioscience

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Abstract

Contactless manipulation of small objects, such as micro-/nanoparticles, biological entities, and even cells is required in varied applications in biosciences. Magnetic levitation (MagLev) is a new-generation methodology to achieve contactless magnetic manipulation of objects. Lately, magnetic levitation methodology has been utilized in several applications in bioscience, such as biosensors, diagnostics and tissue engineering. Magnetic levitation enables separation or positioning of objects in three-dimensional (3D) space based on their density features. Therefore, density-based separation assays utilizing magnetic levitation for biosensing or diagnostic purposes are developed recently. Specific particles or cells, which are markers of any disease, could be detected by sorting them based on density differences through magnetic levitation. On the other hand, tissue engineering studies and production of self-assembled 3D cell culture structures are carried out by magnetic levitation, where cells are magnetically positioned while allowing cell-cell interaction resulting in 3D cell culture formation. Lately, magnetic levitation methodologies received more interest in the field of bioscience due to advantages about the efficiency and cost. This contribution broadly summarizes recent efforts in magnetic levitation techniques that are mainly applied in diagnostics and tissue engineering.

Keywords: magnetic levitation, tissue engineering, diagnostic tools, biosensors, density-based assay, contactless manipulation

1. Introduction

Mimicking the microenvironment of biological systems is crucial, especially for diagnostic and tissue engineering purposes. There are several contact-free manipulation methodologies [1] that mimic and control the microenvironments of biological systems, such as magnetophoresis [2], acoustophoresis [3], electrophoresis [4], and thermophoresis [5]. Magnetophoresis is a method that provides contact-free manipulation of particles in a magnetic field. There is no additional equipment required instead of permanent magnets or electromagnets for magnetophoresis; however, sound waves, electrical source, and heat source are required for acoustophoresis, electrophoresis, and thermophoresis, respectively [6].

Magnetophoresis is a contactless manipulation method, which provides the manipulation of particles in the magnetic field provided by either permanent magnets or electromagnets. During manipulation of particles in a magnetic field gradient or/and in a magnetized medium, neither pH nor temperature of sample is
affected [7]. There are two different types of magnetophoresis, such as positive and negative magnetophoresis (Figure 1A, B). In the positive magnetophoresis, particles that have magnetic properties migrate within nonmagnetic (diamagnetic) medium. On the other hand, particles that do not have magnetic properties migrate within paramagnetic medium in negative magnetophoresis [8, 9]. The migration of particles in both types of magnetophoresis depends on the magnetic susceptibility differences between particle and medium.

Magnetic levitation (MagLev) technique, which works with the principle of negative magnetophoresis, manipulates the diamagnetic particles in paramagnetic medium by providing antigravity conditions (Figure 1C) [10]. The diamagnetic particles that are suspended in a paramagnetic medium are positioned at specific height called levitation height depending on their densities when the external magnetic field is applied. According to magnetic levitation principle, diamagnetic particles are specifically positioned depending on their densities by balancing all forces on particles, which are gravitational force (from gravity) and magnetic buoyant force (from magnetic field).

In magnetic levitation systems, biological entities (as diamagnetic particles) are also levitated and manipulated in a three-dimensional (3D) space as well as nonbiological particles in the paramagnetic medium [11]. The paramagnetic medium is an important parameter for magnetic levitation-based approaches, because magnetic susceptibility differences between paramagnetic medium ($\chi > 0$) and diamagnetic particle ($\chi < 0$) provide magnetic buoyancy force on diamagnetic particles in the presence of external magnetic field [12]. There are different paramagnetic mediums that are used in magnetic levitation-based approaches, such as ferrofluids [13] and paramagnetic salt solutions [2]. Ferrofluids are the suspension of maghemite ($\text{Fe}_2\text{O}_3$) or magnetite ($\text{Fe}_3\text{O}_4$) that has higher magnetic susceptibility than paramagnetic salt solutions; however, observation of the samples is limited because of their opaque nature [14]. Manganese (II) chloride ($\text{MnCl}_2$) and diethylenetriamine-pentaacetic acid (Gd-DTPA) are generally chosen and used paramagnetic agents in magnetic levitation; however, they are used in high concentration to effectively levitate diamagnetic particles because of their low magnetic susceptibilities. The use of paramagnetic salts in high concentration is the main reason for toxicity-based limitations in bioapplications of magnetic levitation technology [2].

![Figure 1. Migration of particles with magnetophoresis. (A) Positive magnetophoresis, which means the migration of magnetic particles in diamagnetic fluid. (B) Negative magnetophoresis, which means the migration of diamagnetic particles in paramagnetic fluid. (C) Migration of diamagnetic particles in paramagnetic fluid via magnetic levitation principle.](image)
1.1 History and theory of magnetic levitation

Magnetic levitation concept appeared within the study named as “An Absolute Micromanometer Using Diamagnetic Levitation” at the end of 1960s. In this study, the diamagnetically levitated ultramicromanometer was described where friction-free suspension was produced via magnetic induction for graphite disk to measure absolute pressure down to $10^{-10}$ Torr [15]. Later, density-based separation was carried out for minerals [16] and metals [17] by magnetic levitation principle. In another study, improvement of magnetic levitation system has been studied to measure the density differences of liquids and solids [18–20]. On the other hand, magnetic levitation-based approaches also appeared in tissue engineering applications. The magnetic levitation techniques were also used to mimic 3D cellular microenvironment and to form 3D cellular structures by guiding the cells [21–26].

As depicted in Figure 2, the diamagnetic particles in paramagnetic medium are density-dependently positioned at specific height, in which each force (gravitational, magnetic buoyant forces, and steric interactions) on diamagnetic particle (Figure 2B) is equalized under magnetic field produced by permanent magnets [Eqs. (1) and (4)] [27–29]. The magnetic and gravitational forces act on diamagnetic particles and cause the particles to either float or sink in paramagnetic solution (Figure 2C) [1].

At balanced height:

$$\vec{F}_g + \vec{F}_m = 0$$  \hspace{1cm} (1)

$$\vec{F}_g = (\rho_p - \rho_m) V \vec{g}$$  \hspace{1cm} (2)

$$\vec{F}_m = \frac{(X_p - X_m)}{\mu_0} V (\vec{B}, \vec{\nabla}) \vec{B}$$  \hspace{1cm} (3)

$$\vec{F}_g + \vec{F}_m = (\rho_p - \rho_m) V \vec{g} + \frac{(X_p - X_m)}{\mu_0} V (\vec{B}, \vec{\nabla}) \vec{B} = 0$$  \hspace{1cm} (4)

In Eq. (4), the gravitational ($\vec{F}_g$) and magnetic ($\vec{F}_m$) forces on levitating particles are balanced. Here, $\rho_p$ and $\rho_m$ refer to the density of levitating particle and paramagnetic medium (kg.m$^{-3}$), respectively; and $X_p$ and $X_m$ represent the

![Figure 2.](image)

The principle of magnetic levitation. (A) Magnetic field between the magnets, which is oriented in anti-Helmholtz configuration. (B) the forces on levitating objects within the magnetic levitation systems. (C) the alignment of levitating objects at specific levitation heights depending on their densities.
magnetic susceptibility of levitating particle and paramagnetic medium (unitless), respectively. \( V \) is the volume of levitating particle (m\(^3\)); \( g \) is the gravitational constant (9.81 m.s\(^{-2}\)); \( \mu_0 \) is the permeability of free space (1.26 \( \times \) 10\(^{-6}\) kg.s\(^{-2}\).A\(^{-2}\)); and \( B \) is the magnitude of applied magnetic field (T).

\[
Z_0 = \left[ \frac{g\mu_0 h^2}{(X_i - X_m)4B_0^2} \right] \rho_p + \left[ \frac{h}{2} - \frac{\rho_m g\mu_0 h^2}{(X_i - X_m)4B_0^2} \right]
\] (5)

According to Eq. (4), the levitation height of levitating particles, which is shown as \( Z_0 \) (m) (Figure 2B), could be mathematically determined in certain space between magnets, which is shown as \( h \) (m) Eq. (5) [28].

2. Magnetic levitation approaches in biosciences

2.1 Magnetic levitation technology for biosensors and diagnostics

Magnetic levitation-based technologies were recently developed for positioning and sorting of diamagnetic particles, which can be either biological or nonbiological materials to be used in biosensing and diagnostic purposes. The magnetic levitation systems in diagnostic field are mainly composed of strong magnets and optical components. N52-grade NdFeB magnets are oriented in anti-Helmholtz configuration where same poles face toward each other. N52-grade NdFeB magnets produce magnetic field around 0.4 T, which causes the magnetic buoyant force on diamagnetic particles suspended in paramagnetic medium [1].

In the earliest version (Figure 3A), magnetic levitation system is fabricated from polydimethylsiloxane (PDMS) material, which is combined with NdFeB magnets. These systems had capability to dynamically separate polymeric diamagnetic particles suspended in paramagnetic salt solution (GdCl\(_3\)) by differentiation of levitation height depending on their density differences. While the diamagnetic particles with highest densities were moved through bottom part of the channel, the diamagnetic particles with lowest densities were moved through upper part of the channel [29]. After that, simple cuvette was integrated into magnetic levitation system instead of PDMS holder to analyze the quality of chemical reactions and also to statically measure the density of diamagnetic solid particles. As shown in Figure 3B, magnetic levitation technique was utilized to monitor chemical reactions by observing the levitation height changes of polymeric beads. The decrease in the levitation heights was observed depending on increasing the density of polymeric beads during solid-phase reaction [28]. Also, the density determination of materials with unknown densities was performed by using density versus levitation height curve, which is produced from materials with known densities [18]. To improve measurable density range that could be measured by magnetic levitation system, “Tilted MagLev” system (Figure 3C) was developed. In such system, the range of measurable densities was expanded around 15-fold; however, the sensitivity of “Tilted MagLev” was limited [19]. To overcome the sensitivity limitations in “Tilted MagLev,” high-sensitivity magnetic levitation setup was developed by rotating “Tilted MagLev.” As shown in Figure 3D, density measurement could be performed with resolution down to 10\(^{-6}\) g/ml, which means the rotated configuration improved the sensitivity 100-fold over previous [20].

On the other hand, a magnetic levitation platform has been developed for analysis of proteins’ binding event. The diamagnetic polymeric beads were covalently functionalized with the derivatives of benzenesulfonamide to be used for capturing
BCA. After capturing protein in solution containing disodium gadolinium (III) diethylenetriamine pentaacetic acid, Gd(DTPA), the changes on the levitation heights of ligand-coated diamagnetic particles were analyzed in magnetic levitation systems. The levitation height of ligand-coated diamagnetic particles changed due to protein capture. Depending on levitation height change of ligand-coated diamagnetic particles, the interaction between ligand and protein, and furthermore protein concentration was determined in the sample [30]. Later, magnetic levitation system was improved for hepatitis C detection. In this system, magnetic levitation principle was combined with enzyme-linked immunosorbent assay (ELISA), called density-linked immunosorbent assay (DeLISA). The surface of diamagnetic
polymeric beads was functionalized with HCV-NS3 protein, and levitation heights were determined before and after anti-HCV NS3 binding. Analyzing the levitation height differences, which are caused by interaction between ligand and protein, the detection of hepatitis C was carried out via DeLISA [31].

Especially for diagnostic purposes, a new-generation magnetic levitation device, which is compatible with light microscope, was designed and developed to analyze micro-sized particles, such as polymeric beads and cells (Figure 3E). This device is composed of two N52-grade NdFeB magnets, microcapillary channel, mirrors and poly(methyl methacrylate) (PMMA) holder. The microcapillary glass channel was integrated in between magnets (oriented in anti-Helmholtz configuration) where all system components were assembled within PMMA holder. The mirrors were placed in 45° angle to reflect light through microcapillary channel for providing the visualization of micro-sized particles within magnetic levitation system. As shown in Figure 3E, the developed system was highly sensitive for density-dependent separation of micro-sized particles and was used to profile densities of different cells, such as breast adenocarcinoma, esophageal adenocarcinoma, colorectal adenocarcinoma, colorectal carcinoma, and red and white blood cells [11]. Later, similar magnetic levitation system was used for diagnosis of malaria-infected red blood cells and sickle cells by analyzing their density-dependent levitation heights [32, 33].

Previously mentioned magnetic levitation systems [11, 32, 33] require highly sophisticated analyzing instruments such as light microscope that causes high cost. To reduce that cost, magnetic levitation system was combined with smartphone to analyze micro-sized particles and even cells. Smartphone-assisted systems allow levitation height determination of micro-sized particles in microcapillary channel by the camera of smartphone and lens, which are used for focusing [34–36]. One of these systems, namely “i-LEV,” was used for density-dependent analysis of cells from whole blood via smartphone. In addition to counting red and white blood cells in whole blood, “i-LEV” could be used in the detection and analysis of single cells where there is a difference between healthy and sick cell. Based on the density-dependent alignment of cells, “i-LEV” was used for monitoring sickle cell disease [35].

### 2.2 Magnetic levitation technology for tissue engineering

Magnetic levitation systems have been also specialized to provide contactless manipulation of cells for the production of 3D cellular structures in tissue engineering applications. In the earliest version, magnetic force-based tissue-engineering (MagTE) setup was developed, which utilizes magnetic cationic lipidosome (MCL)-guided cells for the formation of 3D string or ring-shaped tissue structures under magnetic field provided by permanent magnets. MCLs in the culture medium were uptaken by myoblast C2C12 cells, and then labelled myoblast C2C12 cells were manipulated by inducing the magnetic field provided by permanent NdFeB magnets. The oriented skeletal muscle tissues, string-like and ring-like assemblies, were obtained [21]. Later, 3D bioassembler, which is M13 phage-based hydrogel containing magnetic iron oxide (MIO) and gold nanoparticles (Au/NP), was utilized for the formation of 3D cellular structures via magnetic levitation technology. The cells in magnetic levitation system interacted with each other and formed 3D clusters after 30 min right after cells were levitated. Varied 3D cellular structures were obtained by incubating clustered cells for 24 h. The different cell types, human glioblastoma cells and human astrocytes, were also 3D cocultured [22]. In another study, poly-L-lysine cross-linked MIO/AuNP hydrogel, termed as NanoShuttle, was developed and used in magnetic levitation system to form 3D cellular structures in 96-well plate from varied cell types, for example human
embryonic kidney cells, mouse fibroblast cells, human mammary epithelial, human umbilical vein endothelial cell, and human hepatocyte cells. However, the morphologies of 3D cellular structures were different for each cell line depending on their cellular properties [25]. Also, the same magnetic levitation system was utilized for the formation of multicellular 3D cellular structures, called organoids. The bronchiole wall was formed by sequential layered assembly technique. Epithelial cells (EpiCs), smooth muscle cells (SMCs), pulmonary fibroblasts (PFs), and pulmonary endothelial cells (PEC) were individually grown and levitated for 4 h. Later, levitated cell lines were sequentially taken into bronchiole coculture as EpiCs, SMCs, PFs, and PECs [23]. The same procedure was also applied to form the aortic valve coculture formation from valvular interstitial cells (VICs) and endothelial cells (VECs) [24] and heterogeneous breast tumors from various breast cancer cells and fibroblast [26]. On the other hand, the generation of organoids, called as adipospheres, by the culturing of 3 T3-L1 preadipocyte cells and hEND.3 endothelial cells with magnetic levitation technique has been accomplished [37].

Recently, a new magnetic levitation system, which uses only paramagnetic salt solution instead of NanoShuttles or any other magnetic biomaterial, was developed. Cells were encapsulated into methacrylated gelatin (GelMa) or polyethylene glycol dimethacrylate (PEDGA) hydrogels and they were levitated in the presence of Gd$^{3+}$ solution. 3D cell cultures were achieved by the levitational self-assembly of those microstructures [38].

In tissue engineering applications, the above-mentioned magnetic levitation systems have limitations because of the scaffold or additional compounds were required for 3D cell culturing. The new-generation magnetic levitation system that allows scaffold-free cell culturing was developed to overcome with these limitations [39]. In such system, cells are three cultured in 3D microenvironment without any scaffold materials or additional requirements. The mouse fibroblast cells (NIH 3T3) and non-small-cell lung cancer cells (HCC827) were mixed with Gadolinium (III) (Gd$^{3+}$) chelate, which is a paramagnetic agent, before loading into developed magnetic levitation setup. With the help of paramagnetic agent, the cells were levitated and suspended at their specific levitation height in the presence of external magnetic field. By interacting with levitated cells, cells started to secrete their own extracellular matrixes, and cellular clusters were formed within the magnetic levitation setup. The 3D cellular spheroids were formed from low number of cells ($1\times10^3$) in 48 h; however, the cellular strings were observed when a high number of cells were used ($1\times10^5$) [39].

3. Summary and conclusion

Magnetic levitation-based technologies are new-generation systems that are used in biosensing, diagnostics, as well as tissue engineering. Density-dependent separation of particles provides high-speed analysis of biomolecules, even cells, with low cost in diagnostics. On the other hand, contactless manipulation of cells via magnetic force can also be provided by magnetic levitation systems. In such systems, cells are easily manipulated in the presence of magnetic field after they are magnetized with specialized molecules, such as ferrofluids and paramagnetic agents. By positioning the cells at a specific height in medium, where they can easily interact with each other, natural 3D microenvironment can be formed.

While the magnetic levitation-based systems provide faster, cost-effective, and easy-to-use diagnostic applications, at the same time, it is also used to form 3D cellular structures in tissue engineering field.
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Conflict of interest

The authors declare no conflict of interest.

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