Review

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Magnetic Force-Based Tissue Engineering and Regenerative Medicine

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Among other biomedical applications, magnetic nanoparticles and liposomes have a vast field of applications in tissue engineering and regenerative medicine. Magnetic nanoparticles and liposomes, when introduced into cells to be cultured, maneuver the cell's positioning by the appropriate use of magnets to create more complex tissue structures than those that are achieved by conventional culture methods.

KEYWORDS: *Magnetite Nanoparticles, Magnetic Liposomes, Magnetic Force, Tissue Engineering, Regenerative Medicine.*

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INTRODUCTION

The goal of producing functional tissues is challenged by the complexity of organ architectures. It is difficult enough to vascularize tissue constructs, let alone to recapitulate the complex arrangement of cells and extracellular matrix found in functional organs. The loss of tissue or the failure of an organ is a frequent, devastating, and costly problem in human health care. Tissue engineering (TE) applies principles of biology and engineering to the development of functional substitutes of damaged tissue. In general, tissue engineering involves the following processes:[1]

(a) Target cells are isolated and expanded to the required cell number;

(b) Cells are harvested and reseeded into three-dimensional biodegradable scaffolds, allowing 3D cell culture; and

(c) The cultured 3D constructs are transplanted into patients.

In tissue engineering and regenerative medicine (TERM), production of a scaffold in a mold is an example of a classical and traditional top-down strategy. A more interesting and recent bottom-up approach relies on the self-assembly of a scaffold from smaller components, potentially with different functionalities designed to carry out distinct tasks.

A common characteristic of the current bottom-up strategies for scaffold fabrication is the assembly of molecules into nanoscale structures, which then assemble into macroscopic objects. Self-assembling molecules that form nanoscale structures that then form gels are excellent examples of bottom-up assembly.[2]

Although the technology of these processes in tissue engineering is established, processes can be improved and optimized for specific applications. The use of nanotechnologies has been employed in all the steps of developing for tissue engineering strategies.[3] In particular, such methodologies may be used in physical manipulation of target cells which is essential to advancement in the field of tissue engineering.[4] Treatment concepts based on those techniques would eliminate problems of donor site scarcity, immune rejection and pathogen transfer.[5]

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Nano-sized magnetic nanoparticles and liposomes are increasingly being used across the wide spectrum of the biomedical field including in regenerative medicine. The ability to control the location of these nanoparticles distally using magnets, upon functionalization to enable specific binding, stands to induce a high concentration in a given tissue or organ. Research needs to go beyond using this technique as an investigative tool and should focus on its potential to actively control cellular processes with an eye towards clinical applications, in particular in tissue engineering and regenerative medicine.[6]

MAGNETIC NANOPARTICLES AND LIPOSOMES

The application of nanotechnology has opened a new realm of advancement in the field of regenerative medicine and has provided hope for the culmination of long-felt needs by the development of an ideal means to control the biochemical and mechanical microenviroment for successful cell delivery and tissue regeneration. Both top-down and bottom-up approaches have been widely used in the advancement of this field, be it by improvement in scaffolds for cell growth, development of new and efficient delivery devices, cellular modification and tracking applications or by development of nanodevices such as biosensors.[7]

In the rapidly developing areas of nanobiotechnology and nanomedicine, magnetic nanoparticles (MNPs) are one type of the most well-established nanomaterials because of their biocompatibility and the potential applications as alternative contrast enhancing agents for magnetic resonance imaging (MRI). While developing of MNPs as alternative contrast agents for MRI application has moved quickly to testing in animal models and clinical trials, other applications of biofunctional MNPs, such as manipulating proteins or cells, have been explored extensively at the stage of qualitative or conceptual demonstration.[8] In fact, the response of MNPs to a magnetic field offers a unique way to modulate cellular behavior in a non-contact or "remote" mode, i.e., the magnet exerts force on the cells without direct contact. Procedures to produce biofunctional MNPs have been summarized recently.[9–11]

The high biocompatibility and versatile nature of liposomes have made these particles keystone components in many hot-topic biomedical research areas. Liposomes can be combined with a large variety of nanomaterials, such as superparamagnetic iron oxide nanoparticles (SPIONs).[12] Because the unique features of both the magnetizable colloid and the versatile lipid bilayer can be joined, the resulting so-called magnetoliposomes can be exploit in a great array of biotechnological and biomedical applications. For decades, clinicians have used liposome as nanoscale systems to deliver encapsulated anthracycline molecules for cancer treatment. Instead, the more recent proposition to combine liposomes with nanoparticles remains at the preclinical development stages.[13]

Magnetic cationic liposomes (MCLs) are cationic self-assembled lipid vesicles containing 10 nm magnetite nanoparticles. The cationic surface of MCLs reinforces the electrostatic interaction between MCLs and target cell membranes, resulting in the improvement of the accumulation of magnetite nanoparticles in the target cells. In a typical experimental procedure, the MCLs can be prepared

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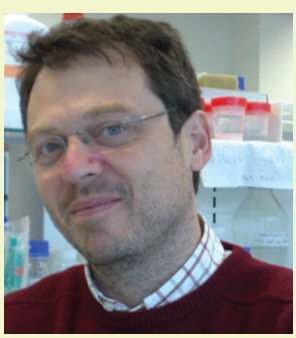

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from colloidal magnetite (10 nm) and a lipid mixture consisting of *N*-(α-trimethylammonioacetyl)-didodecyl-D-glutamate chloride (TMAG), dilauroylphosphatidylcholine (DLPC), and dioleoylphosphatidyl-ethanolamine (DOPE) in a 1:2:2 molar ratio.[1] The mean hydrodynamic size of the magnetic cationic liposomes, determined by dynamic light scattering, was around 150 nm.[14]

In tissue engineering, magnetic separation methods have been employed to isolate target cells in co-culture systems. In these methods, target cells were co-cultured with nontarget cells labeled with magnetite cationic liposomes. Thus, when necessary, the MCL-labeled nontargeted cells were magnetically removed from the co-culture, resulting in negative isolation of the target cells. In these co-cultures, target cells were separated with 94% purity and 98% recovery yield on average.[15] For tissue engineering purposes, also magnetic nanoparticles and magnetic force have been used to concentrate the retroviral vectors to enhance the transduction efficiency and to enable their magnetic manipulation. Additionally, the magnetically labeled retroviral vectors can be directed to the desired regions for infection by applying magnetic fields, and micro-patterns of gene-transduced cell regions could be created on a cellular monolayer using micro-patterned magnetic concentrators.[16]

MAGNETS AND MAGNETIC FORCES

The biomedical application of magnetic forces has long been studied. For example, magnetic resonance imaging is a mainstay of clinical diagnostic radiology, relying on superconducting magnets and large magnetic fields. Magnets have also been used to levitate biological samples through the natural diamagnetism of organic materials. Incorporation of superparamagnetic iron oxide nanoparticles has further enabled manipulation of surface patterns, contrast-enhanced magnetic resonance imaging, cell sorting, mechanical-conditioning of cells, studies of mechanic-sensitive membrane properties, and cellular micromanipulation. SPIONs can also be modified to target proteins or can be coupled to cationic liposomes for delivery and concentration.[17]

Three different geometries are founded among the magnets used for magnetic force-based tissue engineering and regenerative medicine: cylindrical, rectangular, and ring shaped. All of them are permanent magnets consisting of a Neodymium–Iron–Boron alloy (NdFeB) or an Aluminum–Nickel–Cobalt alloy (AlNiCo). Developed in the 1980s, neodymium magnets are the strongest type of permanent magnets made, producing significantly stronger magnetic fields than other types (such as AlNiCo magnets). The magnetic field typically produced by NdFeB magnets can be in excess of 1.3 T, whereas AlNiCo magnets typically exhibit fields between 0.5 and 1 T. The magnets were inserted into a thermo-contractive Teflon tube and both ends of the tube were sealed with a silicone rubber to avoid oxidizing by the culture medium. The magnets were sterilized by exposure to ultraviolet (UV) radiation.[18]

An electromagnet was also used to get a variable magnetic field by Shimizu et al.[19] They obtain a maximum magnetic field intensity of 0.45 T at the tip of the probe that was controlled by a foot switch.

The spatial distribution of magnetic nanoparticles can be distally managed by magnetic forces, a unique possibility for the fine control of the position and the activity of the nanoparticles, and can eventually attach materials without the need of *in situ* direct manipulation. Such a distant control permits precise interventions in complex media or biological systems that could not be reached with alternative approaches.[20] Magnetic nanoparticles are straightforwardly used in magnetic force-based tissue engineering and regenerative medicine (Mag-TERM), by which it is possible to define the position of different cell types in elaborated tissular structures such as multilayered or tubular entities.

MAGNETIC FORCE-BASED CELL CULTURE AND CO-CULTURE TECHNIQUES

Since cells labeled with magnetic nanoparticles (MNPs) and liposomes (MCLs) can be manipulated using magnets, a novel tissue engineering methodology using magnetic force and functionalized magnetic nanoparticles namely, magnetic force-based tissue engineering (Mag-TE) was proposed in 2004 by Ito et al.[21] They showed that nanoparticle-labeled endothelial cells maintained their viability and capacity to form a confluent endothelial monolayer. Moreover, Mironov et al.[22] proposed in 2008 a definition of magnetic force-based tissue engineering: *the biofabrication of more complex tissue constructs using cells, cellular monolayers, and cell aggregates labeled with magnetic nanoparticles.*

Such MCL-labeled cells can be manipulated and organized by magnetic forces while maintaining their functionality (indicating that MCLs are not toxic). In this Mag-TE approach, a magnet is under the culture plate, attracting and accumulating magnetically labeled cells.[23] This allows populations of MCL-labeled cells to be sequentially driven to the surface to create 2D patterned or even 3D multilayered structures, as already tested with several cell lines, including human umbilical vein endothelial cells,[24] retinal pigment epithelial cells,[25] and keratinocytes,[21] among others, with promising results. Mag-TE can be divided into two processes:

(i) Labeling cells magnetically using magnetic nanoparticles or magnetic cationic liposomes, and

(ii) Manipulating magnetically labeled cells directly using a magnetic field.

MCLs were used to magnetically label human keratinocytes. Ito et al.[21] showed that magnetically labeled keratinocytes were accumulated on the surface of the culture plate using a magnet, and stratification was promoted

by a magnetic force to form a sheet-like 3D construct. The addition of MCLs to human keratinocytes resulted in the rapid uptake of magnetite nanoparticles, and the amount of MCLs accumulated in the keratinocytes reached a maximum of 70% of the total added MCLs. Magnetically labeled keratinocytes were seeded and cultured at room temperature into 24-well ultra-low-attachment plates, with a covalently bound Poly(*N*-isopropylacrylamide) hydrogel layer, which is hydrophilic and neutral charged. A neodymium magnet was placed under the plate. Keratinocytes without MCLs, or with MCLs in the absence of a magnet, did not attach onto the plates. In contrast, in the presence of the magnet, the keratinocytes with MCLs accumulated evenly throughout the wells.[26]

Ito et al.[21] also investigated the procedure for harvesting keratinocyte sheets constructed by Mag-TE. The magnet positioned at the reverse side of the plate was removed. Then, a hydrophilic-treated polyvinylidene fluoride (PVDF) membrane was placed on the top of a cylindrical magnet, and the ensemble was positioned on the top interface of the culture medium with the air. Due to the magnetic force, the keratinocyte sheets floated up to the surface of the culture medium without disruption and stuck to the PVDF membrane.

Furthermore, Mag-TE permitted to fabricate and harvest cell sheets that contained HepG2 as a hepatocyte model or NIH3T3 cells as a stromal fibroblast model, as well as heterotypic, layered co-cultures containing different cell lines, as rat hepatocytes and human aortic endothelial cells (HAECs), providing a proof-of-concept for the applicability of this approach for generating complex heterogeneous tissues.[27] Moreover, tubular structures (for example, urinary tissue formed by urothelial cells, smooth muscle cells, and fibroblasts) can also be created using the Mag-TE protocol. In this approach, magnetically labeled cells formed a cell sheet onto which a cylindrical magnet was rolled, which was removed after the tubular structure had been formed[28] (Fig. 1).

Also, angiogenic cell sheets were fabricated using a combination of two magnetic force-based techniques: magnetofection and magnetic cell accumulation. A retroviral vector encoding an expression cassette of vascular endothelial growth factor (VEGF) was labeled with MCLs, to magnetically attract the particles onto a monolayer of mouse myoblast C2C12 cells. MCL-mediated infection increased transduction efficiency by 6.7 times. During the fabrication of the tissue constructs, MCL-labeled cells were accumulated in the presence of a magnetic field to promote the spontaneous formation of a multilayered cell sheet. VEGF gene-engineered C2C12 (C2C12/VEGF) cell sheets, constructed using both magnetic force-based techniques, were subcutaneously transplanted into nude mice. Histological analyses revealed that on day 14 the C2C12/VEGF cell sheet grafts had produced thick tissues, with a high-cell density, and promoted vascularization.[29]

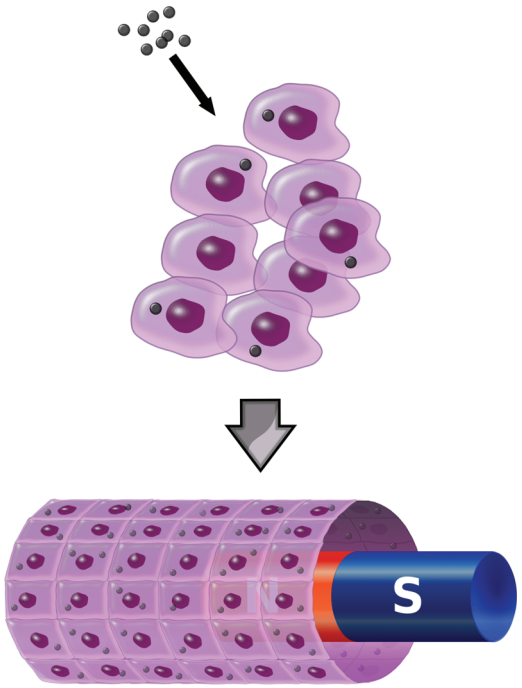

Figure 1. Tubular structures can be generated by folding preformed cell layers, obtained around rod-shaped magnets. Such tubular constructs are recovered after removal of the magnet.

Ito et al.[4] extended the Mag-TE technique towards 3D; in this case a magnetic force was used to precisely place magnetically labeled cells onto target cells and to promote heterotypic cell–cell adhesion to form a three-dimensional construct. Human aortic endothelial cells were magnetically labeled using MCLs, and the labeled HAECs were positioned onto a rat hepatocyte layer using a magnetic force. When a magnet was placed under the culture plate, HAECs accumulated on the hepatocyte monolayer that expressed albumin under thick HAECs layers. In the presence of a magnetic force, layered co-cultures maintained a high level of albumin secretion throughout the study.[30]

Using Mag-TE techniques, Ino et al.[29] successfully fabricated skin-like structures of transgene-expressing fibroblasts and keratinocytes, which indicates that MCLs are a potent biomanipulation tool for 3D tissue construction. Mag-TE was applied for construction of 3D multilayered cell sheets without scaffolds. The cells, magnetically labeled with MCLs, are seeded onto ultra-low-attachment dishes. Subsequently, a magnet is placed under the dishes to accumulate the magnetically labeled cells, and then 3D multilayered cell sheets were constructed.[29] This technology permitted to construct small diameter vascular tubes consisting of hetorotypic layers of endothelial cells, smooth muscle cells and fibroblast.[22] Mag-TE technique can be applied to construct a vascularized Normal Human Dermal Fibroblast (NHDF) sheets incorporating perycites or smooth muscle cells for vascular stabilization after transplantation.[22]

Mag-TE has already been applied to bone tissue engineering: Bone Marrow Stromal Cell (BMSC) sheets without any scaffolds were constructed and transplanted into a rat cranial bone defect, enhancing new bone formation at the defect.[14] Also, Mag-TE was applied to bone tissue engineering using 3D scaffolds. It was also shown that Mag-TE is suitable for the fabrication of skeletal muscle tissue due to characteristics such as scaffold-free tissue engineering, high cell density, and freedom as to the size and shape of the tissue to be fabricated.

Magnetic Cell Patterning

Manipulation of cell patterns in three dimensions in a manner that mimics natural tissue organization and function is critical for cell biological studies and likely essential for successfully regenerating tissues—especially cells with high physiological demands, such as those of the heart, liver, lungs, and articular cartilage.[31] Although recent progress in surface chemistry has enabled the spatial control of cell adhesion onto substrates, conventional methods usually require specialized devices and time-consuming processes to fabricate the substrate. For instance, cell-patterning of mouse fibroblast NIH3T3 cells on a monolayer of HaCaT cells was successfully achieved using poly(ethyelene glycol) to vary the hydrophilicity of the culture substrate and magnetic forces.[32]

Alternatively, Ino et al.[33,24] developed a novel cell-patterning procedure using magnetic cationic liposomes which were designed to improve the accumulation of the 10 nm magnetic nanoparticles into target cells for magnetic cell manipulation. The MCL-labeled cells could be micro-patterned using magnetic field concentrators, in which magnetized micron-thick steel plates were embedded. On the other hand, as the scaffold-free 3D tissue construction approach, multilayered cell sheets were also created by strongly depositing MCL-labeled cells on the culture surfaces by magnetic force. In principle, using the magnetic cell manipulation technique, cell patterns can be created irrespective of surface conditions. By manipulating the strength, shape, and orientation of the magnetic field, multi-directional cell arrangements can be produced *in vitro* and even directly *in vivo*.[34]

Akiyama et al.[35] applied a magnetic force-based tissue engineering technique to cardiac tissue fabrication. A mixture of extracellular matrix precursor and cardiomyocytes labeled with magnetic nanoparticles was added into a well containing a central polycarbonate cylinder. With the use of a magnet, the cells were attracted to the bottom of the well and allowed to form a cell layer. During cultivation, the cell layer shrank towards the cylinder, leading to the formation of a ring-shaped tissue that possessed a multilayered cell structure and contractile properties. The major advantage of the Mag-TE technique is the induction of cell-dense tissues mimicking native tissues, as demonstrated in the fabrication of cardiomyocyte,[40] myoblast cell sheets[36] and muscle tissues.[34]

Okochi et al.[37] fabricated a 3D cell patterning culture system using an external magnetic force and a pin holder, which enables the assembly of the magnetically labeded cells on the collagen gel-coated surface as array-like cell patterns, resulting in the development of a 3D *in vivo* culture model. The cells embedded in type I collagen showed a compacted, spheroid like configuration at each spot, and distinct, accelerated cell growth was observed in cancer model cells compared with control cells. The developed 3D cell culture array was applied to the susceptibility assay of the GM6001 matrix metalloproteinase (MMP) inhibitor, a collagenase inhibitor; a distinct suppression of cell proliferation was observed, while little change was observed in 2D.

Ino et al.[38] also have developed single cell culture arrays using the same procedure. The pin holder was made from magnetic soft iron and contained more than 6000 pillars on its surface. The pin holder was placed on a magnet to concentrate the magnetic flux density above the pillars. NIH3T3 fibroblasts that were labeled with MCLs were seeded into a culture dish, and the dish was placed over the pin holder with the magnet. The magnetically labeled cells were guided on the surface where the pillars were positioned and allocated on the arrays with a high resolution. Single-cell patterning was achieved by adjusting the number of cells seeded, and the target cell was collected by a micromanipulator after removing the pin holder with the magnet. Okochi et al.[39] have fabricated a 3D multicellular tumor spheroid culture array using a magnetic force-based cell patterning method, analyzing the effect of stromal fibroblast on the invasive capacity of melanoma.

Magnetic Cell Seeding

Cell seeding is the first step in constructing 3D tissue-like structures. Although high density cell seeding into scaffolds enhances 3D tissue formation (i.e., cartilage, bone, and cardiac tissue), effective and high-density cell seeding into scaffolds is difficult to achieve. Technical difficulties in cell seeding are caused by the insufficient and inhomogeneous migration of the cells into the overall scaffold volume due to the tortuous path of the interconnected porous structure and by the loss of culture medium with cells out of the scaffold. This prolongs the culture period because of the shortage of initially seeded cells. Therefore, numerous methodologies for effective cell seeding into 3D scaffolds have been investigated, but novel techniques are also required.

In conventional cell seeding (static-seeding), the cell suspension is seeded into small scaffolds using small volumes of highly concentrated cell suspension. The inevitable problem is that the seeded cell suspension flows away with the medium and few cells remain in the scaffolds[40] (Fig. 2(a)).

The use of magnetic forces can facilitate cell seeding into the deep internal space of scaffolds, resulting in higher scaffold-seeding efficiencies. The magnetic force could attract the magnetically labeled cells to prevent them from

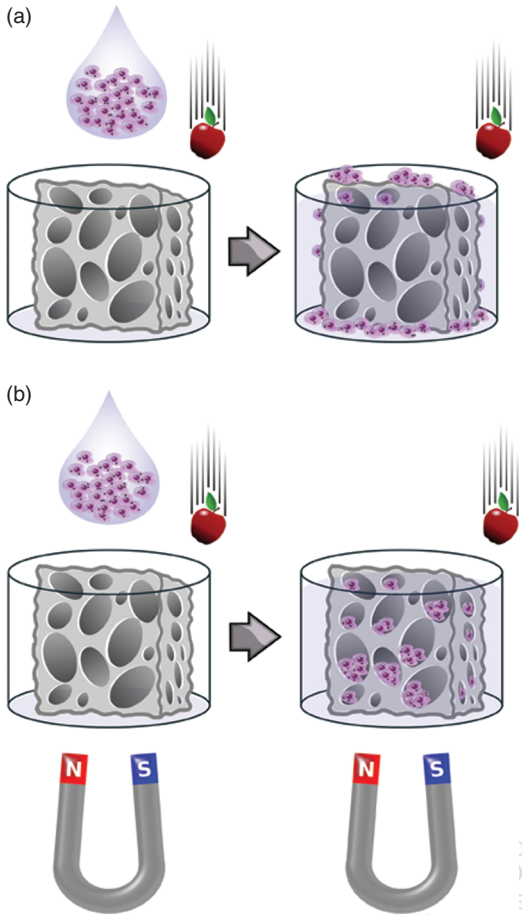

Figure 2. **Scheme presenting the cell seeding process of a scaffold placed at the center of a culture plate. A magnet was placed on the reverse side of the plate and magnetically labeled cells were seeded onto the scaffold. The cells were attracted by the magnetic force, resulting in a high cell seeding efficiency and high cell density scaffold (b), whereas only a low efficiency and a low density were achieved when no magnet was placed (a).**

flowing away[40] (Fig. 2(b)). It was shown that magnetic forces dramatically accelerated cell seeding, cell adhesion, and monolayer assembly.[22]

It has been widely recognized that cells are seeded onto only the superficial layer of three-dimensional scaffolds in tissue engineering technology. To solve this issue, an effective cell seeding technique into the central part of 3D scaffolds is required. Sasaki et al.[41] developed magnetic nanoparticles coated with chitosan for enhancing cellular invasion using magnetic force. Cell-invasion efficiency was enhanced by introducing MNPs into cells and by the presence of magnetic force. The invasion efficacy depends on the intensity of the magnetic force. Matrix metalloproteinases and adhesion molecules that were upregulated in response to the attached nanoparticles and exposure to a magnetic force, may also play a crucial role in improving cell-invasive ability in this system. This current system can efficiently enhance cell seeding into the depth of the scaffold, increase subsequent cell–cell interactions and shorten the period of cell proliferation.[42] This technology seems to be a useful and effective strategy for vascular and bone tissue engineering.[4] However, its application *in vivo* should be examined further before clinical applications, especially in terms of safety issues.

Magnetic Cell Levitation

Souza et al.[18] proposed a three-dimensional tissue culture based on magnetic levitation of cells in the presence of a hydrogel base of filamentous bacteriophase containing gold nanoparticles (Au NPs) and superparamagnetic iron oxide nanoparticles. By spatially controlling the magnetic field, the geometry of the cell mass can be manipulated, and multicellular clustering of different cell types in co-culture can be achieved. The methodology is based on the cellular uptake and subsequent magnetic levitation of a bioinorganic hydrogel. Incorporation of SPIONs creates a new material that retains the biocompatibility of gold-phage hydrogels while adding capabilities for the culture and magnetic manipulation of cells (Fig. 3).

The technology reported provides an alternative to the use of biodegradable porous scaffolds and protein matrices. This methodology is cost effective, because it does not require a specific medium, and it is compatible with standard 2D cell culture techniques. In fact, magnetically levitated human glioblastoma cells showed similar protein expression profiles to those observed in human tumour xenografts. Control of culture shape, and the ability to bring cultures together for controlled interaction in a confrontation assay with *in situ* monitoring, has been demonstrated.[43] This simple, flexible and effective magnetic cell levitation technology may be suitable for a range of applications in biotechnology, stem cell research, and regenerative medicine. Indeed, a potential long-term goal

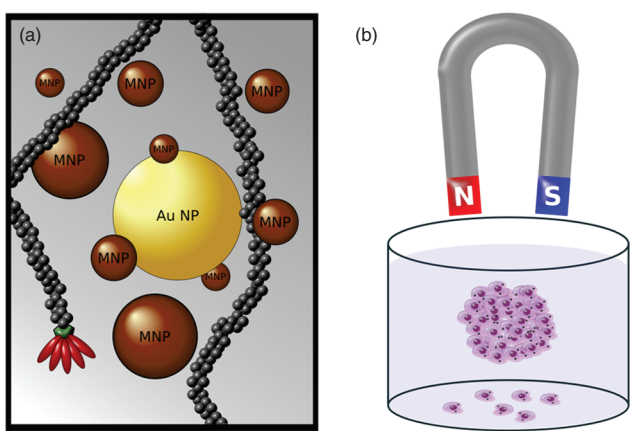

Figure 3. **Magnetic iron oxide containing hydrogels (a) and human glioblastoma cells treated with magnetic iron oxide-containig hydrogel held at the air-medium interface by a magnet (b).**

is the possibility of accomplishing the engineering of normal tissues or complex organs.

The magnetic cell levitation method, which has been explored by Nano3D Biosciences (Boston, USA) in the form of the Bio-Assembler™ devices, provides the advantages of 3D cell culturing in a platform that is no more complicated than standard 2D culturing. The Bio-Assembler™ uses biocompatible polymer-based reagents to deliver magnetic nanoparticles to individual cells so that an applied magnetic field can levitate cells off the bottom of the culture dish and bring cells together near the air-liquid interface. This initiates cell–cell interactions in the absence of any artificial surface or matrix. Magnetic fields are designed to rapidly form 3D multicellular structures in as little as a few hours, including expression of extracellular matrix proteins, which is must faster than any other 3D cell culture technique. The morphology, protein expression, and response to exogenous agents of resulting tissue show great similarity to *in vivo* results.[43]

All cell types that have been tested with the Bio-Assembler™ have been cultured successfully, including human cell lines (glioblastomas, astrocytes, fibroblasts, adipocytes, and endothelial cells), stem cells (murine neural, mesenchymal, and dental pulp stem cells), and primary cells (endothelial, smooth muscle, epithelial, fibroblasts, chondrocytes, and cells isolated from adipose tissue).

We thought that the example of the commercialized Bio-Assembler™ system is perhaps the most compelling and intriguing of those presented. Nowadays only a few examples of applications where it has been tested, particularly *in vivo*, can be found in the scientific literature, enlightening and providing strong support for the potential utility of magnetic cultures and cellular structures. Daquinag et al.[44] used a 3D levitation tissue culture system based on magnetic nanoparticle assembly to model white adipose tissue development and growth in organoids termed adiposheres. They showed that 3T3-L1 preadipocytes remain viable in spheroids for a long period of time, while in 2D culture, they lose adherence and die after reaching confluence. Upon adipogenesis induction in 3T3-L1 adipospheres, cells efficiently formed larger lipid droplets typical of white adipocytes *in vivo*, while only smaller lipid droplet formation is achievable in 2D. Lee et al.[45] demonstrated that hydroxyapatite could selectively be distinguished from various calcium salts in human aortic smooth muscle cells *in vitro* and in calcified cardiovascular tissues, carotid endarterectomy samples and aortic valves, *ex vivo*, by only evaluating, at an early stage, the mineralization process induced by external stimuli, osteogenic factors and a magnetic suspension cell culture.

CONCLUDING REMARKS

The future of cell culturing for biomedical applications lies in the creation of multicellular structure and organization in three dimensions. Many schemes for 3D culturing are being developed or marketed, such as bio-reactors or protein-based gel environments, but they suffer from high cost, low throughput, poor scalability, complexity, or the presence of non-human biological factors that can alter cell behavior and preclude therapeutic uses.

However, the labeling of mammalian cells with magnetic nanoparticles allows the fabrication of complex tissues that are not achievable by conventional cell culture and co-culture, such as 2D and 3D cell layers, tubular tissues, or ordered 3D assemblies consisting of several cell types. The proven lack of toxicity of MNPs and their progressive development into *in vivo* applications is expected to provide exciting tools in the near future for *in situ* manipulations, in which cells could be magnetically distributed for precise tissue engineering.

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