WHITEPAPER

Magnetic 3D cell culturing – A simple and effective technology with a wide range of applications

Cell culture is an essential tool in drug discovery, tissue engineering, stem cell research, as well as in basic research. Presently, two-dimensional (2D) monolayer cell culture is still predominant, but suffers from accuracy issues; for example, attrition rates of drug candidates for cancer were approximately 95 % in clinical trials, which suggests the inadequacy of preclinical in vitro testing [1]. These shortfalls can be traced to the use of conventional 2D cultures, where extracellular matrix (ECM) components, cell-to-cell and cell-to-matrix interactions are not sufficiently expressed or present. These characteristics are important for differentiation, proliferation and cellular functions in vivo [2]. This limitation has led to the development of in vitro 3D cell culture techniques designed to provide a more physiologically relevant cellular environment that can potentially improve accuracy in research and drug discovery [3-5].

Here we describe effective tools for 3D cell culture: the magnetic levitation method and the magnetic bioprinting (Fig. 1). The core technology is the magnetisation of cells with NanoShuttle™-PL. These magnetised cells can then be aggregated with magnetic forces, either by levitation or printing, to form structurally and biologically representative 3D models in vitro.

With magnetised spheroids, liquid addition and removal is made easy by using magnetic forces to hold spheroids in position, thereby limiting spheroid loss. Spheroids can also be picked up and transferred between vessels using magnetic tools such as the MagPen™ [6].

NanoShuttle™-PL, small nano particles approx. 50 nm in diameter, consists of gold, iron oxide, and poly-L-lysine [7]. NanoShuttle™-PL magnetises cells by electrostatically attaching to cell membranes during an overnight static incubation. After incubation, magnetised cells will appear peppered with dark nanoparticles. NanoShuttle™-PL will stay attached to the cell membrane for up to 8 days, at which point it is released into the 3D culture [8]. NanoShuttle™-PL is biocompatible, having no effect on metabolism, proliferation, and inflammatory stress [6,7], and even encouraging proliferation in 3D [8,9]. Additionally, it does not interfere with experimental techniques, such as fluorescence [9] or western blotting [11].

Magnetic Levitation

Magnetic levitation is an easy tool to create native tissue environments in vitro. Cells are magnetised with NanoShuttle™-PL through overnight incubation and dispensed into a cell-repellent dish or multiwell plate, where they are levitated off the bottom by a magnet above the cell-repellent vessel [9]. In levitating cells off the stiff well bottom, the magnetic forces work as an invisible scaffold that rapidly aggregates cells to encourage cell-cell interactions and induce ECM synthesis. The resultant 3D culture is formed without any specialised media and can be cultured long-term [10].

Applications of Magnetic Levitation

The magnetic levitation method has been successfully used to make 3D cultures with different cell types, including cell lines, stem cells and primary cells [6,9,10-14]. The basic application of this technology is to culture 3D cell cultures under different environmental conditions, and then analyse them using common biological research techniques, such as immunohistochemical analysis [6,10] and western blotting [11]. For example, magnetic levitation was used to create an invasion assay between two separate cultures of human glioblastoma and normal astrocytes to investigate the mechanisms of glioblastoma invasion (Fig. 2) [9,11].

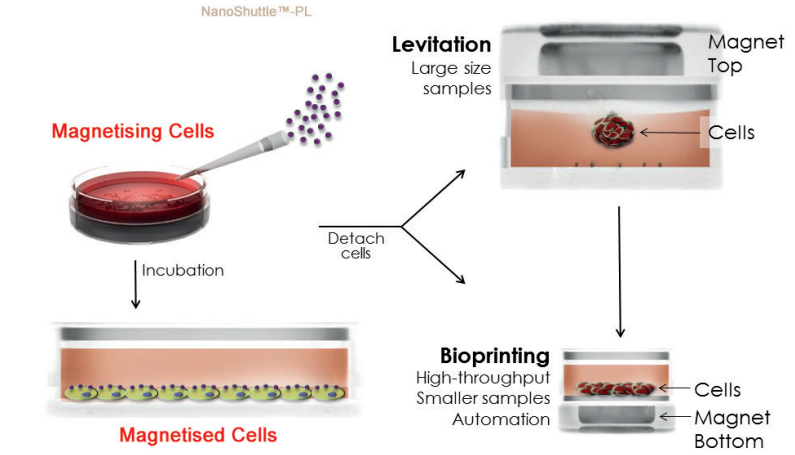

Figure 1: Magnetic 3D bioprinting and levitation

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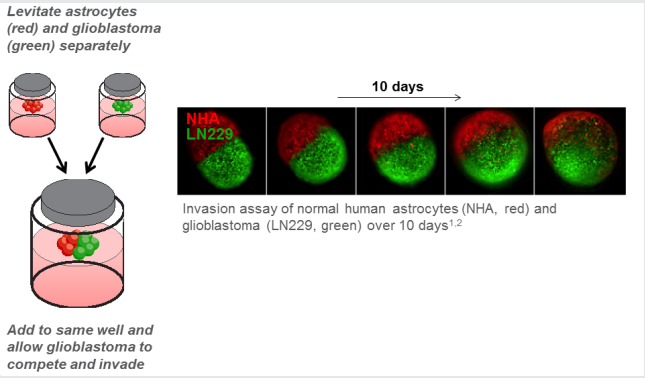

Figure 2: Invasion assay of human glioblastoma [9,11]

Also the magnetic levitation has been used to differentiate stem cells in 3D; 3T3-L1 pre-adipocytes were differentiated into adipocytes and formed a vascularised adiposphere in co-culture with endothelial cells (Fig. 3) [10,13]. Such 3D cultures can be created in petri dishes or 6 and 24 well plates with cell-repellent surfaces.

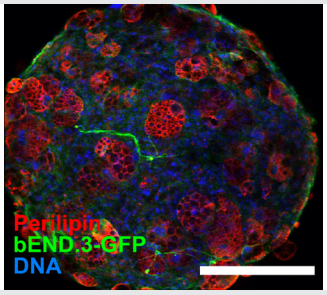

Figure 3: Spheroid formed by 3T3-L1 preadipocytes and GFP-expressing mouse bEND.3 endothelial cells. Post-adipogenesis spheroids were subjected to immunofluorescence with perilipin antibodies (red) and GFP antibodies (green) [10].

Magnetic Bioprinting

In contrast to magnetic levitation, in magnetic 3D bioprinting, cells incubated with NanoShuttle™-PL overnight are printed into spheroids by placing a 96 well plate full of magnetised cells atop a drive of magnets. The magnets below the well aggregate the cells using mild magnetic forces to form a spheroid at the bottom of the well. After 15 min to a few hours, the plate containing the spheroids can be removed from the magnetic drive and cultured long-term. These spheroids escape the limitations of other platforms in high-throughput screening by being rapidly formed, unattached to any stiff substrate, reproducible in size with fixed magnets, unlimited to any cell type, and scalable in size for high-throughput formats (96 and 384 well).

Applications of the Magnetic Bioprinting

With magnetic 3D bioprinting viable spheroids can be printed that grow over time, and the viability of cells in these spheroids can be assessed continuously using commercially available assays like RealTime-Glo™ (Promega). Such cell viability assays, together with the 3D printing method, provide an ideal combination for high-throughput compound screening [15]. Additionally, it was demonstrated that magnetic 3D bioprinting can be used to develop novel migration assays, and that automated kinetic imaging helps to facilitate high-throughput and high-content screening. These assays form a foundation for robust screening of compound effects on cell migration (Fig. 4) [16].

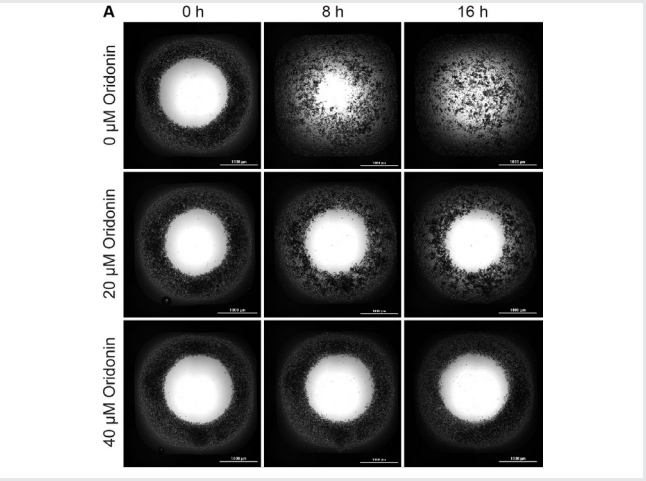

Figure 4: Keratinocytes were successfully printed into 3D rings with magnetic 3D bioprinting, where the closure of center holes/wounds can be imaged in the Cytation™ (BioTek Instruments). Exposure to oridonin notably stopped ring closure with increasing concentrations.

The Perfect Match for Magnetic Cell Culture

Magnetic cell culture is advantageous because it facilitates the rapid formation of spheroids, easy handling and avoids attachment of the spheroids to a stiff substrate that can affect cell behavior. Rather than use standard tissue culture vessels, Greiner Bio-One CELLSTAR® cell culture vessels with cell-repellent surface can effectively prevent cell attachment and therefore provide the perfect match for magnetic cell culturing.

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Revision: July 2016 - F073 774

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