A Novel System Enabling High Efficiency Low Toxicity Transfection of Cells in 3D Culture

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Mirus The Transfection Experts

Abstract

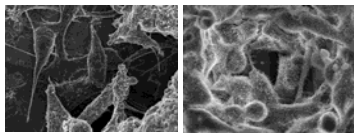
Three-dimensional (3D) cell culture is an emerging trend in cell biology as it permits complex multicellular organization that more closely mimics the mechanical and biochemical cues present in native tissues when compared to routinely used two-dimensional (2D) cell culture. Transfection is an advantageous technique frequently used by cell biologists in 2D cell culture and requires further development to be effectively expanded to 3D cell culture.

In this study, we demonstrate a transfection system that implements the alvetex® polystyrene scaffold and a transfection formulation that enables high efficiency transfection of cells that have been adapted and grown in 3D culture. Unlike current 3D transfection protocols, which require transfection of cells in suspension prior to plating in 3D matrices, we have established a system whereby cells are effectively transfected within the scaffold. Various transfection reagent formulations were tested for efficacy in terms of reporter output, e.g. luciferase and green fluorescent protein (GFP) expression, and absence of cytotoxicity in 3D culture. To facilitate acclimation of cells to 3D culture, critical parameters such as cell density and post-seeding adaptation time were fine-tuned to obtain optimal transfection efficiencies. Transfection performance was further improved by titrating DNA dosage and reagent-to-DNA ratios. Optimization of cell growth conditions and transfection parameters has led to the development of a novel high efficiency, low toxicity transfection system specific for 3D cell culture.

Advantages of 3D Cell Culture

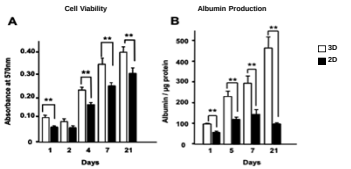
Three-dimensional (3D) culture aims to provide for the growth of cells in spatial environments which promote optimal growth, differentiation and function in the laboratory.

Cells grown in 3D in alvetex® display enhanced differentiation and organization compared with counterparts grown in 2D, maintaining a 3D shape and form close interactions with adjacent cells.



2D cell growth **3D alvetex® cell growth**

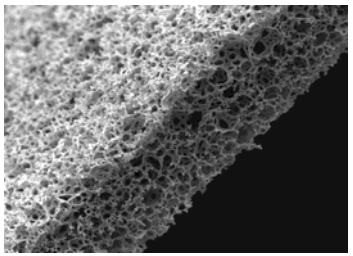
Scanning electron microscopy images of HepG2 cells grown in 2D culture versus alvetex® scaffold. Cells in alvetex® grow more homogeneously and develop a 3D form characteristic of liver tissues in the body. *Bokhari et al. J Anat. 2007, 211(4), 567-576*

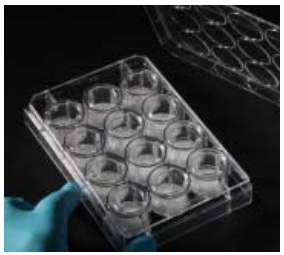


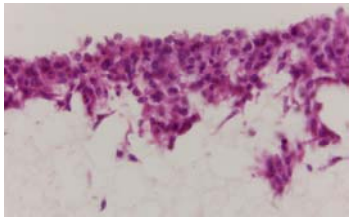
Cell viability was determined using a MTT assay and showed greater numbers of viable HepG2 hepatocyte cells in alvetex® than on 2D substrate. Secretion of albumin from 3D HepG2 cells was elevated compared to 2D culture. Data has been normalized to total protein to take into account differences in cell numbers.

There is increasing requirement and demand for *in vitro* systems that more accurately mimic *in vivo* tissue environments. Cells maintained in 3D typically exhibit enhanced functionality compared with those cultured on traditional 2D surfaces. As 3D culture technologies become more integral for research, a wide range of applications including cell-based assays, and molecular biology techniques such as transfection need to be developed specifically for 3D matrices.

3D Cell Culture Technology





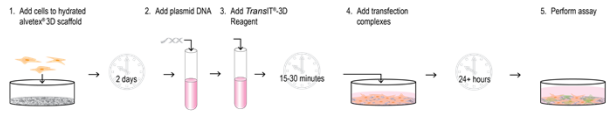


HepG2 cells seeded onto alvetex® 12 well plates and cultured for 7 days.

alvetex

- Allows individual cells to maintain their normal 3D shape and structure with minimal exogenous support and interference.
- Enables a more natural environment to foster the creation of native architecture found in tissue structures.
- Reduces stress and artificial responses as a result of cell adaptation to flat, 2D growth surfaces.

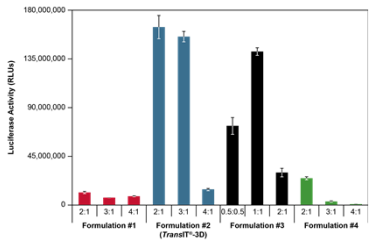
Culture and Transfection Overview



Experiments were performed by seeding cells into 12 well alvetex® 3D plates, then allowing cells to adapt to the 3D scaffolds for a period of days. On the day of transfection, complexes were formed by mixing plasmid DNA and transfection reagent in Opti-MEM. After transfection complex incubation of 15-30 minutes, complexes were added to scaffolds containing cells. After at least 24 hours, cells were harvested and assayed for appropriate reporter.

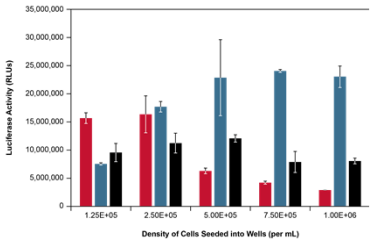
Optimization of Transfection Conditions

A. Novel transfection formulation testing



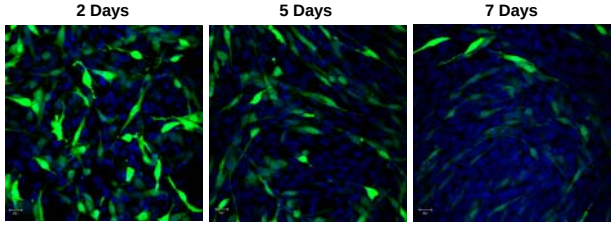
Four potential transfection reagent formulations were screened in HepG2 cells. Alvetex® plates were seeded at the optimized density 48 hours before transfection. Cells were transfected by mixing the transfection reagent with a plasmid encoding firefly luciferase at the indicated reagent-to-DNA ratios. Luciferase activity was measured at 24 hours post-transfection. Formulation #2 (*Trans*IT ® -3D) showed highest expression and least variability between ratios.

B. Cell density at the time of seeding



HepG2, CHO-K1, or NIH3T3 cells were seeded at the indicated densities in 12 well alvetex® 3D plates and adapted to 3D culture conditions for 48 hours. Following adaptation, cells were transfected with *Trans*IT®-3D (as represented in A) combined with a plasmid encoding firefly luciferase at the reagent-to-DNA ratio of 3:1. Luciferase activity was measured 24 hours post-transfection. Seed density affects expression levels and optimal density must be determined empirically for each cell line.

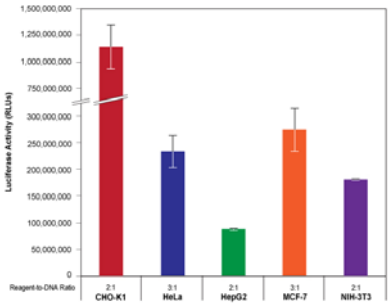
C. Post seeding adaptation time



NIH3T3 fibroblast cells were seeded at optimized cell density in alvetex® 3D plates and adapted to 3D growth for the indicated time points. After adaptation, cells were transfected with *Trans*IT®-3D combined with a plasmid encoding Green Fluorescent Protein. Cells were stained with the nuclear stain Hoechst 33342 (blue) and visualized via confocal microscopy. Optimal post seeding adaptation is 48 hours.

High Expression Transfection in 3D Culture

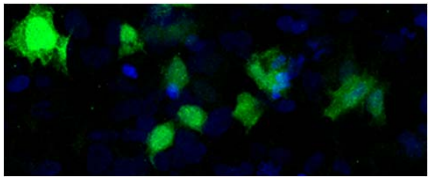
*Trans*IT®-3D Transfection of Multiple Cell Types



Five commonly used cell lines were seeded at optimized cell densities in 12-well alvetex® 3D plates and adapted to 3D culture conditions for 48 hours. After adaptation, cells were transfected with *Trans*IT®-3D combined with a plasmid encoding firefly luciferase at the reagent-to-DNA ratios indicated. Luciferase activity was measured 24 hours post-transfection. Expression levels vary between cell lines, but each has been successfully transfected.

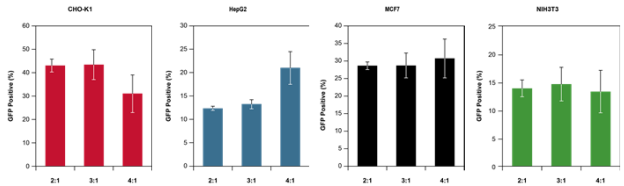
High Efficiency Transfection in 3D Culture

A. Expression of GFP assayed via confocal microscopy



HepG2 cells were seeded at the optimized cell density in alvetex® 3D plates and adapted to 3D growth for 48 hours. After adaptation, cells were transfected with *Trans*IT®-3D combined with a plasmid encoding Green Fluorescent Protein at a 3:1 ratio of transfection reagent-to-DNA. Cells were stained with the nuclear stain Hoechst 33342 (blue) and visualized by confocal microscopy. Cells within scaffolds have been transfected, illustrating the ability of *Trans*IT®-3D to penetrate into 3D matrices.

B. Transfection efficiency measured by flow cytometry



Cells were seeded at optimized cell densities in 12-well alvetex® 3D plates and adapted to 3D culture conditions for 48 hours. After adaptation, cells were transfected with *Trans*IT®-3D combined with a plasmid encoding Green Fluorescent Protein at the reagent-to-DNA ratios indicated. Efficiency of delivery was measured 24 hours later (CHO-K1 and NIH3T3) or 48 hours later (MCF7 and HepG2) using a BD LSRII flow cytometer. Efficient plasmid delivery is observed in multiple cell lines in various ratios.

Conclusions

- A novel transfection formulation was discovered that facilitates transfection in a variety of mammalian cells grown in 3D culture.
- High protein expression and high transfection efficiency can be attained in 3D culture using *Trans*IT®-3D.
- Cells grown in alvetex® scaffolds more closely mimic an *in vivo* environment than cells grown on 2D surfaces.
- Minimal optimization of transfection reagent-to-DNA ratio is required for efficient transfection.