Development and Implementation of a Novel 3D Culture Technology

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Introduction

Specific limitations associated with 2D cell culture have been discerned; such as the loss of tissue-specific architecture, mechanical and biochemical cues, and cell to cell interactions[1, 2]. To address these issues, many are now turning to 3D-based cellular assay systems that permit the formation of multicellular structures (MCS). It has been shown that in contrast to traditional 2D methods, cells cultured in a 3D format may exhibit unique bio-chemical and morphological features similar to their corresponding tissues *in vivo*[3]. Disease models possessing increased clinical relevance may inform and improve therapeutic intervention. We at Vale Life Sciences, based at the Translational Research Institute, have developed a 3D culture technology that is cost effective, scalable, rapidly deployable, user-friendly and compatible with imaging and liquid handling systems, as well as flow cytometry[4, 5]. Happy Cell Advanced Suspension Medium ® (HC) is a low viscosity liquid matrix which promotes the formation of 3D MCS by permitting natural cellular migration, aggregation and proliferation, without restrictions imposed by artificial matrices[4]. HC has also been shown to facilitate the expansion and growth of single cells and enhance the viability of primary cells[6]. When no longer required this suspension system can be inactivated, thereby simplifying cell harvesting and downstream processing. Here, we demonstrate the ability of HC to generate *in vitro* 3D MCS which are compatible with a number of downstream processes.

Methods

HC is supplied as a 4X concentrate and is readily diluted to the required working concentration with standard cell culture medium and desired additives. A 1X concentration is appropriate for most applications. HC is available in a range of culture medium bases and has been designed to sustain cells in long term culture. To use, simply collect cells by centrifugation, re-suspend in HC prepared to the required working concentration, and plate out in low adhesion culture vessels. Using a variety of cell types we have demonstrated its suspension properties, its promotion of cellular viability, and its ability to facilitate the generation of both cellular aggregates and clonal spheroids. Designed to work in conjunction with HC, the complementary Inactivation Solution (IS) facilitates sedimentation and recovery of 3D MCS for downstream processes such as imaging or protein and nucleic acid investigation.

Data

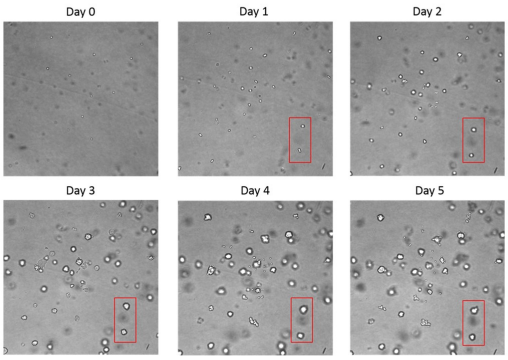

Figure 1: 3D structure formation in HC over a period of 5 days.
(IN Cell Analyzer, 4X magnification).

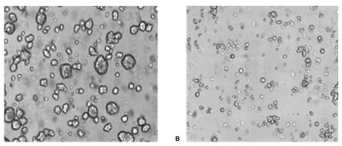

Figure 2: (A) LNCaP and (B) RWPE-1 cells cultured in HC.
(Cytell Imaging System, 4X magnification).

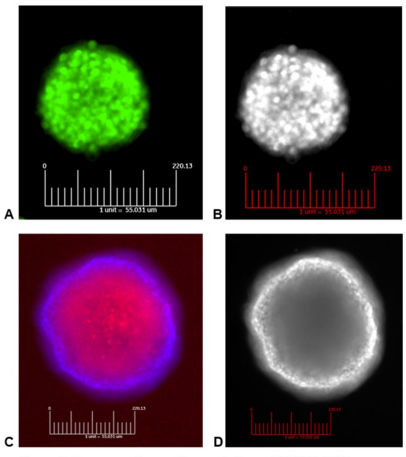

Figure 5: Presence of necrotic core in larger H460 3D MCS
Cyquant viable cell stain, approx.150 µm in diameter (A & B). Hoechst nuclear stain and PI dead cell identifier, approx. 300 µm in diameter (C & D). (Cytell Imaging System, 10X magnification).

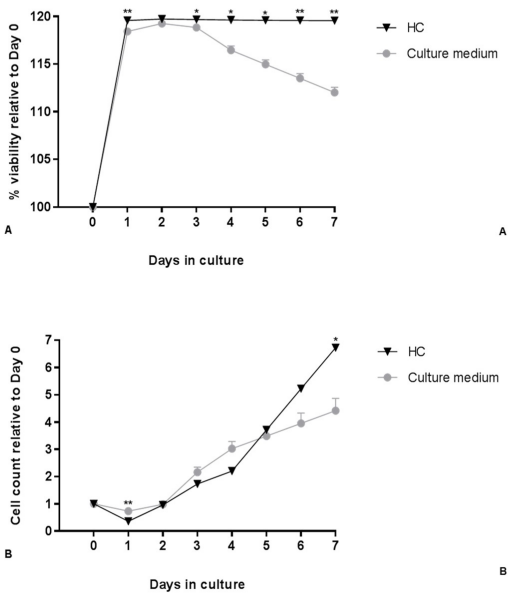

Figure 3: 7 day viability and proliferation assessment of Jurkat cells cultured simultaneously in HC and complete culture medium.
Cellular viability was significantly increased in cells cultured in HC versus those cultured in standard culture medium (A). A significant increase in HC culture proliferation was also observed after a 7 day period ($p<0.05$) (B). Data graphed as mean ± SEM (n=3). Statistical analysis was performed using an unpaired two tailed Welch's *t* test (*$p<0.05$, **$p<0.01$).

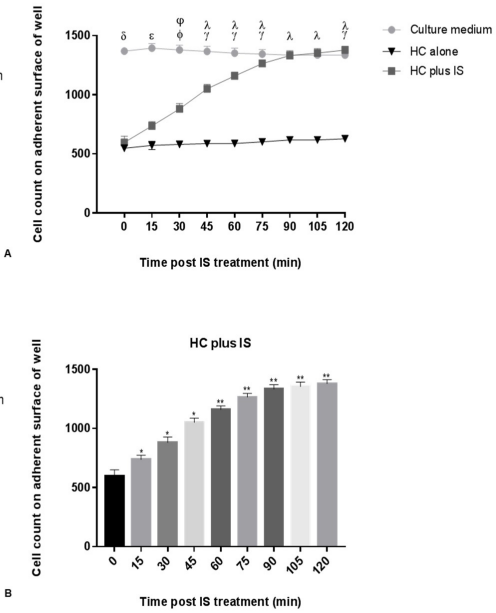

Figure 4: H460 cell count at surface of well after addition of IS.
Mean ± SEM (n=3). A: Unpaired two tailed Welch's *t* test (δ $p<0.0001$ HC plus IS versus complete medium; ε $p<0.001$ HC plus IS versus complete medium; ϕ $p<0.01$ HC plus IS versus complete medium; γ $p<0.05$ HC plus IS versus complete medium; φ $p<0.05$ HC plus IS versus HC; λ $p<0.01$ HC plus IS versus HC). B: Paired two tailed Welch's *t* test (*$p<0.05$; **$p<0.01$ *vs.* 0 min).

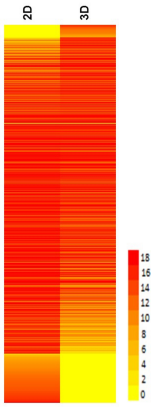

Figure 7: Heat map representation of protein expression in 2D and 3D samples. 1337 proteins represented. Scale:10^6 based on total Intensity Chi^2 analysis.

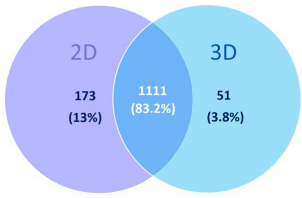

Figure 6: Number of proteins in common and unique to each sample.

Table 1: 2D upregulated pathways
Translation
Cell cycle
RNA processing
Protein localisation
Protein transport
Proteolysis
Macromolecule catabolic process
Macromolecule complex assembly
Regulation of cellular protein metabolic process
mRNA processing
RNA splicing
Cellular response to stress
Cellular macromolecule localisation

Table 2: 3D upregulated pathways
Oxidation reduction
Apoptosis
Macromolecular complex assembly
Proteolysis
Generation of precursor metabolites and energy
Macromolecular catabolic process
Autophagy
Intracellular transport
Response to organic substance
Modification-dependant protein catabolic process

Conclusion

Happy Cell ASM® has been tested and utilised by major pharmaceutical and biotech companies, contract research organisations and academic institutions. It is featured in an ever increasing number of research communications and is a viable alternative to solid gel matrices, scaffolds, micro-patterned surfaces and hanging drop systems for 3D cell culture. For more information please visit www.valelifesciences.com.

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