

A novel hydrogel mountant - CyGEL™ - enables temporospatial HCS imaging assays of live non-adherent cells

Edward R, Errington RJ, Patterson LH, Ogrodzinski S and Smith PJ

Biostat Ltd, 56 Charnwood Road, Shepshed, Leicestershire, LE12 9NP, UK

P8002

Introduction

Imaging-based assays have primarily relied upon adherent cell types for temporospatial measurements. This limits choice of cell type and extension of detection technologies to relevant biological models that may comprise non-adherent (e.g. suspension culture) or indeed 'detaching' (e.g. loss of viability/adherence) cell states such as drug candidate screening in immunological and lymphoproliferative disorder models, where the therapeutic target is often presented by a non-adherent type cell or where a biological response requires changes in adherence to a substrate. New technologies are thus needed to deal with live cell-based HCS assays on non-adherent cells or to exploit suspension cells in fluidic systems.

Here we describe CyGEL™ - a thermo-reversible hydrogel-based 4-D immobilization technology for live cell location without imposing an anchoring to a substrate.

Figure 1: Chilled CyGEL™ dispensed onto microscope slide and coverslip overlaid. Re-chilling the gel allows it to spread

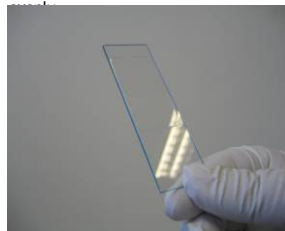


Figure 2: GFP expressing cells mounted in DRAQ5™-doped CyGEL™. DRAQ5™ far-red emitting live cell nuclear counterstain

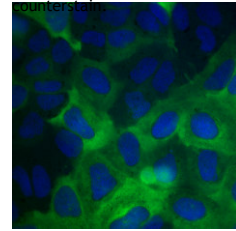


Figure 3: CyGEL™ is compatible with live cells. Adherent cells were imaged before and 10 and 30 min. after being overlaid with CyGEL™.



Key benefits of CyGEL™ for HCS in live non-adherent cells

- Rapid preparation of live cells for gel mounting
- Long term analysis of non-adherent cells
- Acts as a support matrix, distinct from vessel surface
- Sterile, low temperature recovery of viable cells from gel
- Multiple assay formats & applications incl. apoptosis

Technological Features

THERMO-REVERSIBLE: CyGEL™ is a liquid when chilled and rapidly gels above 15°C. Live cells are mixed with cold CyGEL™, warmed to hold them for imaging. Cells can be recovered for further analysis e.g. RT-PCR by re-cooling.

OPTICALLY INERT & COMPATIBLE WITH FLUORESCENCE: CyGEL™ is optically clear and inert with low auto-fluorescence (fig.1). CyGEL™ has a refractive index of 1.365 at 37°C (100% strength, as supplied) similar to water (1.33). Fig.2 demonstrates its compatibility with fluorophores.

COMPATIBLE WITH LIVE CELLS: cells retain viability and morphology while protein expression is unperturbed (Fig. 3). HeLa cells held in CyGEL™ for up to 4 hours retained viability and entered apoptosis in the expected manner (Upton, 2007).

IMMOBILIZES CELLS & BEADS TEMPOROSPATIALLY: allows calibration using beads, and temporo-spatial and high resolution imaging of live cells held in space over time (see fig 4.).

Figure 4: Red fluorescent beads in buffer (top series) and in CyGEL™ (bottom) imaged at t = 0 sec., 1 sec., 2 sec., 3 sec. and a composite. In the composite, white events have not moved, coloured events have moved from frame to frame. The top image used one bead a point of

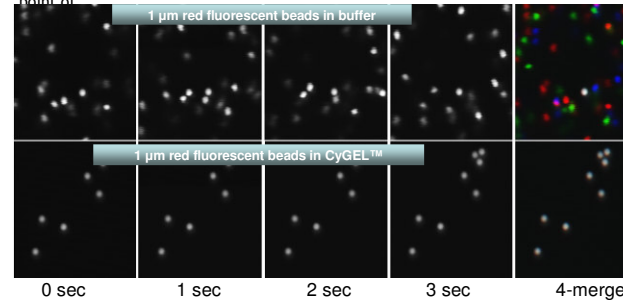


Figure 7: Live cell nuclei imaged in 20µM DRAQ5™-doped CyGEL™.

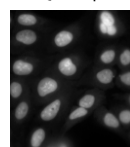
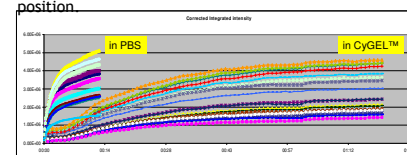


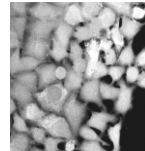
Figure 8: DRAQ5 (20 µM) staining kinetics for individual live tumour cell nuclei imaged in PBS and CyGEL™. The range of steady-state intensity reflects cell cycle position



Application Examples

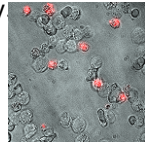
CELL REPORTER ASSAYS: CyGEL™ Clear used to mount cells with internal fluorescent signals (e.g. GFP) or functional probes (e.g. calcein labeled cells, as in fig. 5).

Figure 5



APOPTOSIS ASSAYS: CyGEL™ doped with 1 µM Propidium Iodide (PI) used to observe apoptosis over time. Fig. 6 shows a composite image of lymphoma cells taking up PI as they become leaky

Figure 6



COUNTERSTAIN-LOCALIZED CELLS: CyGEL™ doped with a nuclear counterstain (e.g. DRAQ5™ stained cells, as in fig. 7) suitable for cell cycle position, trans-locations and end-point (antibody-tagged) measurements.

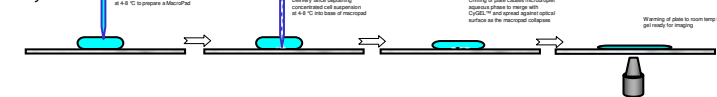
CONTROLLED DELIVERY OF FLUOROCHROMES / DRUGS: kinetics of delivery of small molecules is modified in CyGEL™, where the gel acts as a reservoir. The kinetics of DRAQ5™ staining cells in PBS and CyGEL™ is compared in fig. 8.

Cell Imaging Preparation

Macropad method: Cells and CyGEL™ premixed then deposited



Microdroplet method: Cell droplet injected into CyGEL™



N.B. both of these methods can be used in microplate wells or converted to microarrays.

Conclusions / Future Work

A biologically-compatible mountant, CyGEL™ has advantageous optical properties for fluorescence imaging and tunable physical properties for gel-sol transition and molecular probe delivery as part of HCS protocols. It can support culture media additives, cell-permeant dyes, and other small molecules.

Future work will include methodologies for parasites: trypanosoma, leishmania, plasmodium sp. and whole organism screening: *D. rerio*, *C. elegans*