Automated Imaging-based Screening Assay to Assess Pluripotent Stem Cell Responses to Substrates

In collaboration with Nikon, the Centre for Commercialization of Regenerative Medicine ("CCRM") has developed a **rapid and automated imaging-based screening assay to assess pluripotent stem cells (PSC) responses to substrates** that may be of interest to industry members involved in **developing next generation stem cell maintenance and/or cell culture substrates.**

1 | Market Need

Human pluripotent stem cells (PSCs) have broad applicability for cell therapy and other regenerative medicine applications. Originally derived and cultivated on feeder cells, a variety of substrates are now available that enable feeder-free PSC culture. These feeder-free approaches eliminate risks associated with contamination from xenogenic feeder cells and enhance process standardization.

Screening for novel synthetic substrates that support the maintenance of PSC can be a time consuming process, often requiring laborious cell enumeration/growth assays and cell characterization assays to assess PSC growth and maintenance over time. Here we show proof-of-principle using the Nikon BioStation CT® to assess pluripotency maintenance, however the assay has broad applicability to assessing novel cell culture substrates in general.

2 | Product

- Automated imaging-based screening assay to assess cell adhesion and pluripotency on various substrates

3 | Competitive Advantages

- A non-destructive and automated assay;
- Allows users to acquire temporal data related to cell morphology, adhesion, proliferation and pluripotency with fewer samples than would be needed in conventional assays;
- Absolute cell numbers can be determined by exploiting the exponential relationship between average cell size as a function of time.

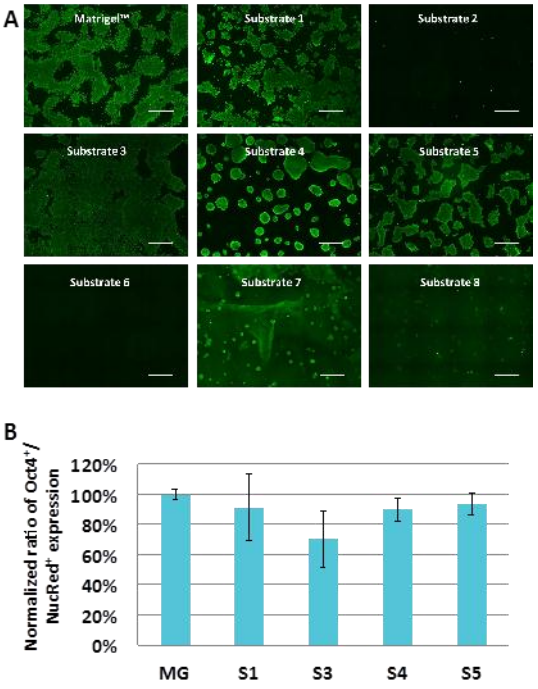

Figure 1. Expression of Oct4 in cells grown on commercial substrates. (A) BioStation CT fluorescence images after Oct4 staining of cells grown for 4 days (scale bars = 500 µm). (B) Normalized ratio of surface area coverage of $Oct4^+/NucRed^+$ cells.

4 | Technology

4.1 Technology Description

The Nikon BioStation CT® is a compact cell incubator and monitoring system that allows users to conduct live cell imaging and facilitates a broad array of long term time-lapse experiments; including studies of cell growth, morphology and protein expression. It provides consistent environmental control of temperature, humidity and O_2/CO_2 concentration in combination with phase and fluorescence imaging. Initial studies suggested that the Nikon Biostation CT can be used as an effective tool to rapidly screen the performance of cell culture substrates.

4.2 Proof of Principle

Matrigel™ and eight other commercially available substrates were prepared in a 48-well plate format. hES2 cells were seeded at 20,000 cells/cm^2 and cell adhesion and proliferation were determined on the BioStation CT and directly compared against results obtained by a CyQUANT® DNA assay (**Figure 2**). Cell adhesion was assessed 24 hours post-seeding and proliferation was

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monitored over a 3 or 4 day period. Cell adhesion results were normalized to Matrigel™ control culture, and fold expansion was calculated on the basis of an increase in cell number or cell area, at a given time-point divided by the same measure at 24 hours. An imaging-based algorithm based on cell area coverage determination was developed for CL-Quant software in collaboration with Nikon. Moreover, a substrate-dependent method of converting cell area coverage to cell number was demonstrated as a means of extrapolating cell numbers from the imaging-based approach. After 4 days of culture, cell seeded substrates were fixed, permeabilized and immunostained for Oct4 expression (**Figure 1**). Pluripotency was determined using ImageJ to analyze the ratio of Oct4 to NucRed™ expression and values were normalized against the Matrigel™ control as a rapid test of relative pluripotency. The **BioStation CT method showed enhanced discrimination between substrates, and proved a suitable tool for the rapid screening of various substrates** for PSC growth and maintenance.

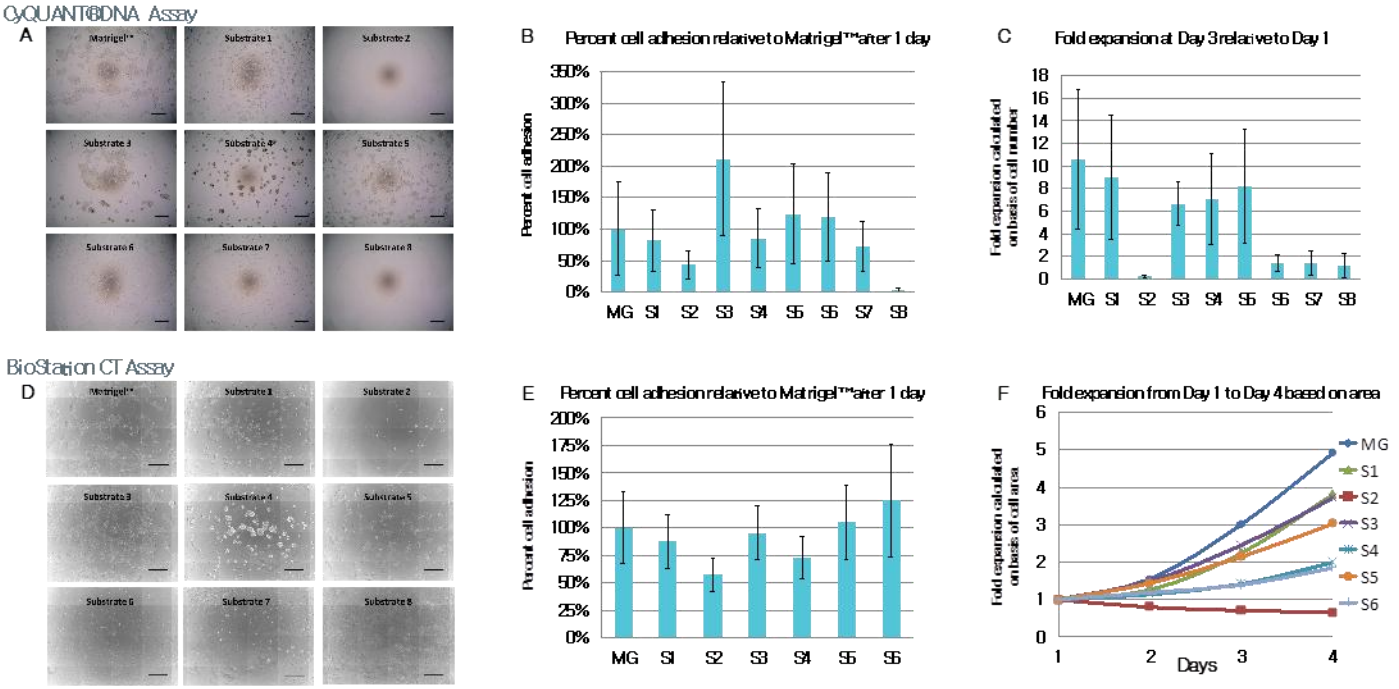

Figure 2. Cell morphology, adhesion and fold expansion determined using the CyQUANT® DNA assay (A-C) and the BioStation CT (D-F). (A) Phase contrast images of hES2 seeded substrates 3 days post-seeding (scale bars = 500 µm). (B) Percent cell adhesion after 1 day relative to Matrigel™ control (N=3). (C) Fold expansion of hES2 cells at day 3 relative to day 1 time-points (N=3). (D) Phase contrast images of hES2 seeded substrates 3 days post-seeding (scale bars = 500 µm). (E) Percent cell adhesion after 1 day relative to Matrigel™ control, determined using CL-Quant software to measure cell area coverage from images (N=3). (F) Fold expansion of hES2 cells from day 1 to day 4, calculated using CL-Quant software to measure the area of cell coverage for images (N=3).

4.3 Intellectual Property

- No IP has been filed for this assay. Nikon owns all rights to the Biostation CT.

4.4 Lead Investigator(s)

- Dr. Patrick Blit at the Centre for Commercialization of Regenerative Medicine, Toronto, Ontario
- Nikon

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