

A RAPID AND SENSITIVE METHOD FOR MEASURING CELL ADHESION

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ABSTRACT

We have adapted the CyQuant® assay to provide a simple, rapid, sensitive and highly reproducible method for measuring cell adhesion. The modified CyQuant® assay eliminates the requirement for labour intensive fluorescent labelling protocols prior to experimentation and has the sensitivity to measure small numbers (>1000) of adherent cells.

INTRODUCTION

The CyQuant® assay (Invitrogen) can be adapted to provide a rapid and sensitive method for measuring adherent cell number. Conventional techniques require cells to be pre-labelled with a fluorescent dye before adherence; this is labour intensive, prone to inaccurate readings due to interference by matrix proteins, and may affect cell behaviour. We have shown that the modified CyQuant® assay is a fast and reliable method that has the sensitivity to detect small numbers of adherent cells (>1000). CyQuant®GR dye is used to selectively label cellular DNA post experimentation and provides a rapid readout of adherent cell number without the time consuming pre-labelling procedure.

RESULTS AND DISCUSSION

Laboratories worldwide investigate cell adhesion to provide functional information on proteins and molecules of interest. Conventional analysis of cell adhesion involves time-consuming cell labelling protocols prior to monitoring cell attachment to basement membrane components such as collagen, fibronectin or a mixture of components, for example, Matrigel™ (BD Biosciences, UK)(1). Protocols that simply stain cells such as crystal violet are non-specific since this dye stains protein as well as DNA (2,3) and will not discriminate between cell and the basement membrane component, potentially leading to a false readout. Methods commonly used to quantitate adherent cells require cells to be labelled with a fluorescent dye, followed by standardization of label uptake per run to ensure an accurate adherent cell number readout. Cellular uptake of the fluorescent dye can vary considerably between experiments, and experiments conducted over longer timeframes are also hampered by leakage of the fluorophore. These labelling protocols require additional time for cell labelling (1 h) and generation of a standard curve of cell number per run (0.5 - 1 h) to determine labelling efficiency. Generation of a standard curve demands a higher cell number input per run ($> 1 \times 10^6$ cells) as well as further time for analysis.

The choice of fluorescent label used has its own limitations. For instance, Calcein AM, a compound that is hydrolysed by intracellular esterases to release fluorescent calcein, is more suited for post-experiment labelling or short duration experiments since the fluorescent signal lasts only 8 hours (4). For increased longevity of signal, the carbocyanines (DiI and DiO) can be used (4-6). These are lipophilic compounds which act by incorporating into the cell membrane but these compounds may also effect cellular electron transport therefore compromising cell integrity (7). Similarly, carboxyfluorescein dyes (CFDA-SE and CFDA-SE) are stable for longer periods and act by covalently binding to intracellular amino groups,

therefore requiring use in amine free buffers and these compounds are also sensitive to changes in pH (4, 8).

To overcome these limitations, we have adapted the CyQuant® assay, to provide a rapid method for measuring cell adhesion with the sensitivity to detect low cell numbers (1×10^3 to 1.5×10^4 cells). We have used this assay to measure adherence of haematopoietic suspension cells (K562) transfected with CCN3. The modified CyQuant® assay utilises CyQuant® GR dye, a strong green fluorescent dye which binds nucleic acids. CyQuant® will detect DNA only and therefore does not give interference from matrix components. In addition, this method is rapid and does not involve labour intensive cell labelling and standardization per run, reducing cell number input and handling time.

The basic protocol is as follows: Once cells have been in contact with the matrix for the required timeframe, non-adherent cells are washed off and the plate is frozen for at least 30 min at -70°C (or up to 4 weeks). The plate is then thawed, cells are lysed with buffer containing CyQuant® dye for 5 minutes and the fluorescence read at 520 nm (excitation 480 nm, emission 520 nm). Fluorescence is proportional to DNA content or cell number and is unaffected by the presence of Matrigel™ (Figure 1A). To determine if CCN3 expression altered K562 cell adhesion, cells transfected with CCN3 (5×10^4) and cells transfected with empty vector (5×10^4) were plated onto Matrigel™ and allowed to adhere for 24 h. CCN3 expression increased the capacity of K562 cells to adhere to Matrigel™ (Figure 1B) (Mean fluorescence for control $11,678 \text{ AFU} \pm 1092$ and CCN3 $30,314 \text{ AFU} \pm 2853$; $n=3$, $p=0.008$). Expression of CCN3 resulted in a 3-fold increase of adherent cells (Mean cell number for control 4740 ± 615 and for CCN3 15225 ± 1605 , $n=3$, $p=0.008$).

The CyQuant method is fast, sensitive and highly reproducible. Since the cells are lysed they are non-viable for use in other experiments, and thus it is an endpoint assay that

measures adherent cell number. If it is important to monitor cells in real time, alternative assays are available that can accomplish that goal (CDFDA-SE). The modified CyQuant® assay is a less labour intensive method for measuring adherent cell number and is as cost effective as other fluorophores that are commonly used. Since this method is suited for measuring low cell numbers, it could be particularly beneficial for use with primary human cells.

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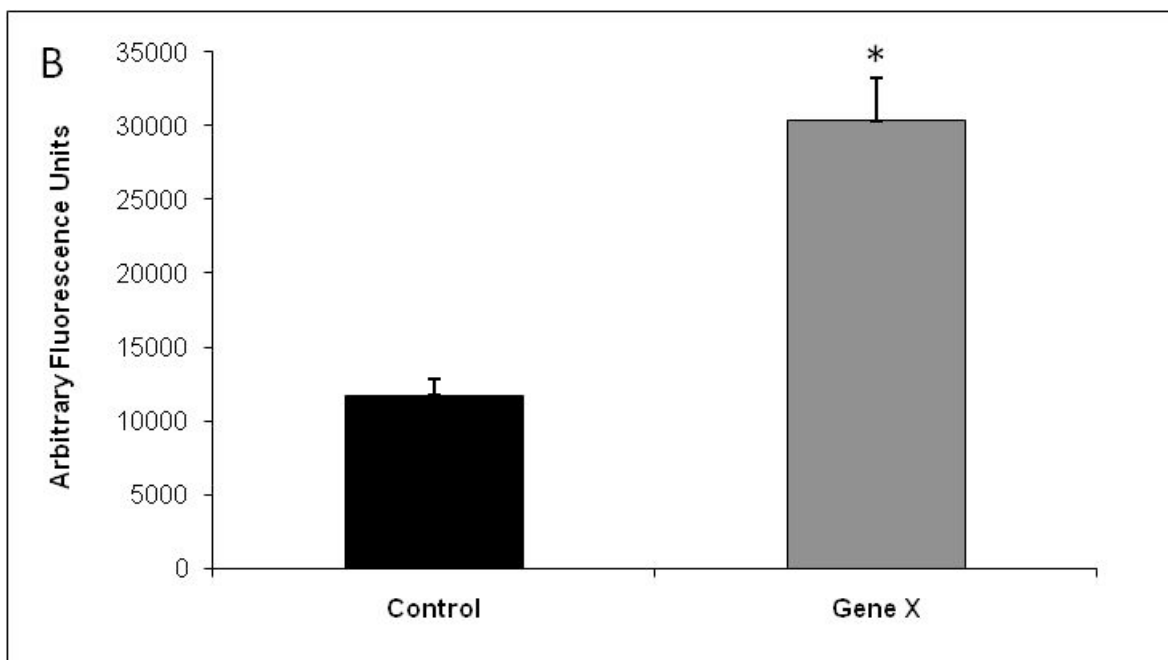
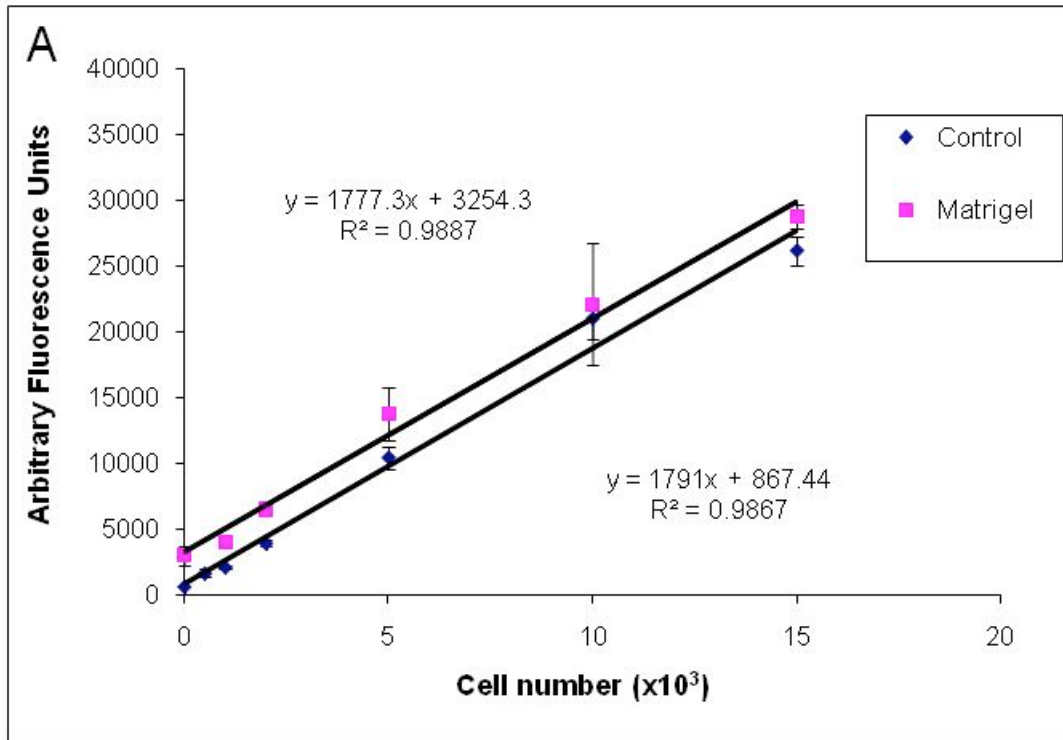
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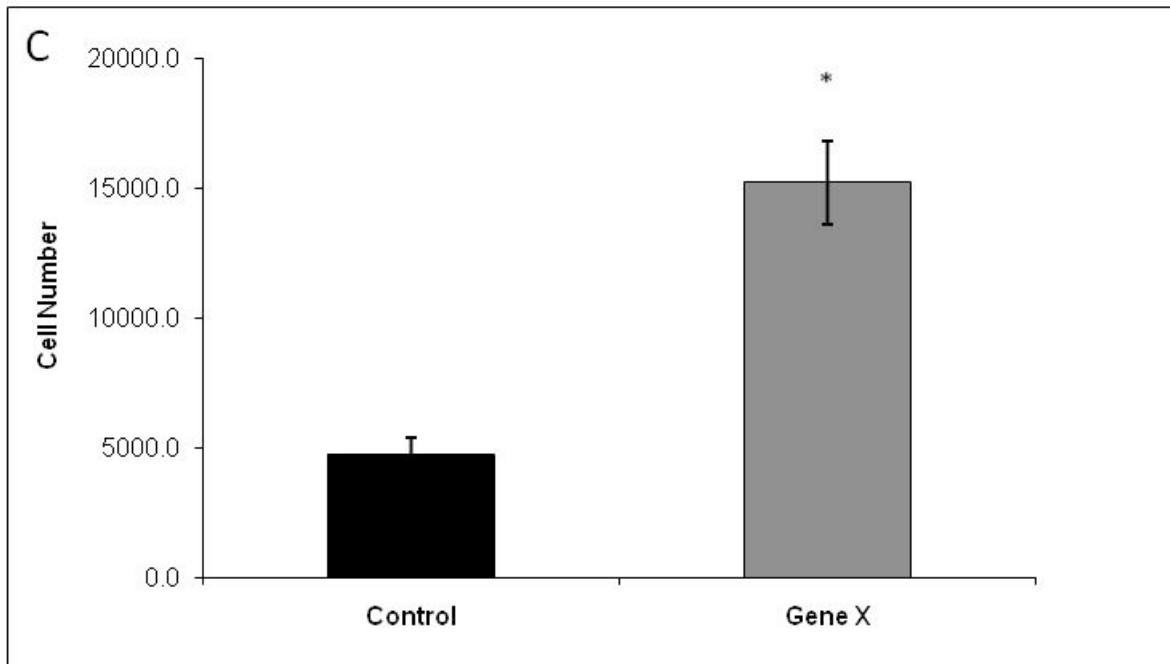
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FIGURE 1





Legend for Figure 1: CyQuant® assay is a rapid method for monitoring cell adhesion.

K562 suspension cells were used to evaluate the CyQuant® assay as a measure of cell adhesion using untreated cells, cells transfected with CCN3 and cells transfected with empty vector (Control). (1A) K562 cells (1×10^3 - 1.5×10^4) were titrated onto 96 well tissue culture plates and Matrigel™ coated plates. CyQuant® assay was performed and fluorescence plotted against cell number to identify a linear relationship that is unaffected by the presence of Matrigel™. (1B) Cells (5×10^4) were plated onto Matrigel™ and allowed to adhere for 24 h. Fluorescence generated from adherent cells is plotted for control cells and cells expressing CCN3 (Gene X). (1C) Cells (5×10^4) were plated onto Matrigel™ and allowed to adhere for 24 h. The number of adherent cells is plotted for control cells and cells expressing CCN3 (* $p=0.008$, $n=3$; shown as Gene X).