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Research Paper

The impact of transient heat transfer on tissue culture cell distribution

Jeffrey D. Brown [a,*,1,2], Heather E. Dillon [b,2], Dorcas V. Kaweesa [b], Arden M. Harada [a]

[a] Department of Biology, University of Portland, 5000 N Willamette Blvd, Portland 97203, OR, USA
[b] Shiley School of Engineering, University of Portland, 5000 N Willamette Blvd, Portland 97203, OR, USA

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It is common practice to subculture adherent eukaryotic cells in multi-well plates at room temperature before 37 °C incubation for growth. Under these conditions, cell distribution was non-uniform with a higher density of cells near the edges of the wells. Non-uniform cell density can alter cell behaviour in numerous biological assays and can result in variability when using automated plate readers on intact cells. This study investigated the possibility that the non-uniform cell distribution was caused by temperature gradients in the growth medium and well walls. Cell density analyses revealed significantly greater cell densities near well walls. Temperature distribution was documented using infrared imaging and temperature-sensitive LCD films, and a transient heat transfer mathematical model was developed to characterise the system and compared to the cell density results. The model predicts that an initial less-than-1 °C temperature gradient is present in the well shortly after initiating 37 °C incubation, leading to preferential cell adhesion to the warmer edges of wells in the first ~30 min of incubation. Techniques to remediate non-uniform cell distribution were evaluated, and a simple method proved effective to promote uniform cell densities across wells of 24-well plates.

1. Introduction

Adherent eukaryotic cells are often cultured in multi-well polystyrene plates with 6–96 wells. A top view of a typical 24-well plate containing a dilution series of stained cells is shown in Fig. 1. The individual wells are separated from adjacent wells by an air gap, and loose-fitting lids allow gas exchange but prevent bulk air movement around individual wells over these plates. Cells are typically seeded into wells at room temperature before incubation at 37 °C in a humidified chamber. In spite of mixing to avoid cell pooling, cell distribution is often observed to be non-uniform, with a higher density of cells near the edges of the well walls.

Many cell behaviours are density dependent. For example, cell proliferation and gene expression (Tanaka, Nakao, Sekimoto, Oka, & Yoneda, 2013) and epidermal growth factor

* Corresponding author.
E-mail address: jbrown6@linfield.edu (J.D. Brown).
[1] Present address: Linfield College Department of Biology, 900 SE Baker St., McMinnville OR 97128, USA.
[2] These authors contributed equally to this work.
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Nomenclature

α	thermal diffusivity ($m^2\ s^{-1}$)
β	volumetric thermal expansion coefficient (K^{-1})
Bi	Biot number
C_n	coefficient of the infinite series solution to the transient heat diffusion equation
Fo	Fourier number
h	heat transfer coefficient ($W \cdot m^{-2} \cdot K^{-1}$)
k	thermal conductivity ($W \cdot m^{-1} \cdot K^{-1}$)
Pr	Prandtl number
R, r	radial coordinate (m)
r^*	dimensionless radial coordinate
Ra	Rayleigh number
θ^*	dimensionless temperature
t^*	dimensionless time
υ	kinematic viscosity ($m^2 \cdot s^{-1}$)
ζ_n	eigen values for the positive roots of the transcendental equation

(EGF) responsiveness (Takahashi & Suzuki, 1996) are dependent on cell density. Therefore, non-uniform cell density across wells can result in variable cell responses in numerous biological assays. In addition, assays of intact cells using automated plate readers are sensitive to variation in cell density, and modest differences in the cell density of the sampled regions of wells can contribute to significant errors: areas with higher cell density yield correspondingly stronger signals.

While inhomogeneous cell distribution is commonly observed when using multi-well plates, this lack of uniformity is typically ignored by investigators and has not been systematically examined in the literature. Suggestions to promote uniform cell distribution have been informally passed among investigators. These strategies include maintaining cells and reagents at 37 °C culture temperature during the entire cell seeding procedure, incubating cells at room temperature for an extended period of time before shifting to a 37 °C culture incubator, or flooding regions between wells with buffer or media. It is interesting to note that each of these proposed solutions alters the dynamics of temperature change across the culture well as cells adhere to well bottoms after seeding. Given this common thread, we sought to document whether thermal gradients exist across plates and their individual wells when used in the traditional manner. Further, we sought to assess the efficacy of methods to promote uniform cell distribution across wells of common 24-well plates.

It is not surprising that temperature differences across a culture space might impact cell distribution. Indeed, robust cell–cell and cell–substrate adhesion of animal cells are temperature dependent (Moscona, 1961). While weak initial adhesion and cell aggregation can occur at 2 °C (Takeichi, 1977) and in formaldehyde-fixed cells (Attramadal & Jonsen, 1974), cytoskeleton-reinforced adhesion cannot be established under these conditions (Angres, Barth, & Nelson, 1996). Investigators probing temperature-dependent adhesive behaviours typically tested adhesion of cells to glass or tissue-culture-treated/hydroxylated polystyrene plates (Curtis, Forrester, McInnes, & Lawrie, 1983) at refrigerated (4 °C), room (22 °C) and body (37 °C) temperatures. These investigations typically revealed a ~50% reduction in adhesion rate at room temperature compared to body temperature (Sagvolden, Giaever, Pettersen, & Feder, 1999); however, robust adhesion was eventually achieved at the lower temperature. Given the large temperature differences in these studies, previous data seem to suggest that small differences

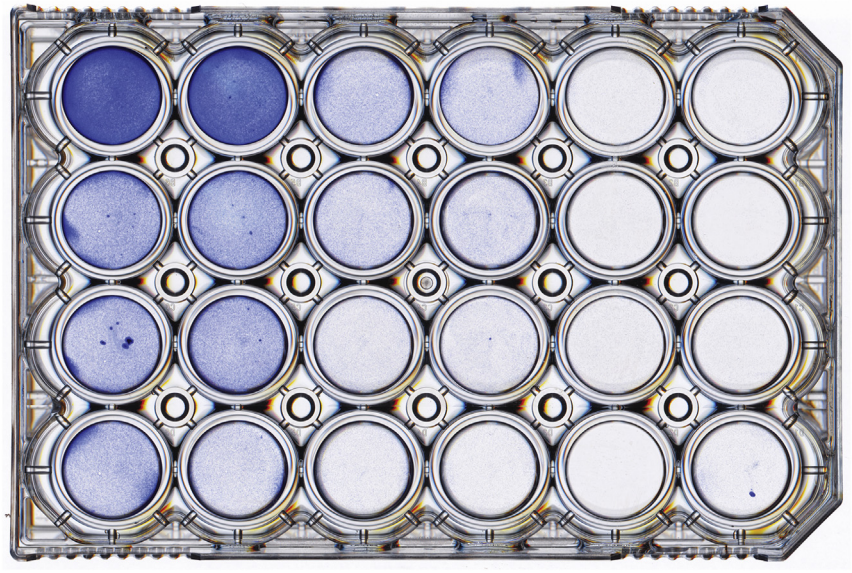

Fig. 1 – Methylene blue stained L929 cells. A serial dilution series of mouse L929 fibroblasts was plated ~24 h before fixing and staining with methylene blue. Lower density wells are near the bottom right corner of the plate. Darker staining was observed near the edges of individual wells (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).