Increasing Cell Adherence with a Hypoxia Workstation

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Abstract

A major criticism of all-in-one workstations (sometimes known as incubated workstations or incubated glove boxes) over using traditional incubators and biosafety cabinets for cell culture is that the exposure to excessive vibration will affect cell growth and adhesion. Here we tested the cells' capabilities to attach in a Baker Ruskinn SCI-tive hypoxia workstation as compared to a standard CO_2 incubator with and without rubber feet (or ferrules) attached to the interior floor tray. The floor tray with all 15 ferrules did remarkably better than a standard incubator, increasing cell adhesion by 25% over a standard incubator. This was confirmed by measuring the reduction in vibration in each planar direction. The floor tray with ferrules significantly reduces vibration exposure to the cells and increases adhesion, both critical for cell growth.

Introduction

Current cell culture methods involve perpetual incubation in a warm environment. Incubators are the most prevalent tool; however, workstations are becoming more popular. A workstation provides a constant environment for cultures, allowing all media changes and potential imaging to be done in-house, without cells being exposed to cooler room air. This is most relevant when working with hypoxia. Maintaining a constant hypoxic environment without exposure to normoxia has been shown to be extremely beneficial to cultures, including stem cells (Kay et al., Regenerative Medicine, 2015).

However, a common concern is the amount of vibration that cells are exposed to from the integral fans when housed in one of these environments, leading to possible detrimental effects on the adherence of *in vitro* cell cultures. In this study, adhesion of monolayer cells inside a standard incubator was compared to a SCI-tive hypoxia workstation. SCI-tive is a class of closed cell culture incubated workstations in which the atmosphere continuously recirculates, with user-defined O_2 and CO_2 levels. These workstations are typically used for long-term hypoxic cell culture, during which it is important that cells are kept in as close to *in vivo* conditions as possible. One concern with incubators is the amount of vibration reaching the culture dishes or flasks, which affects cell adhesion. Here we show that internal vibration of SCI-tive is not significant.

Figure 1. *SCI-tive (left) with inset showing ferrules that support the internal floor tray (right).*

To quantify cell adhesion, cells labeled with Calcein were plated and fold-change in adherence was quantified relative to a control (adherence in an incubator). Cell adhesion assays were then performed in both a standard CO_2 incubator and in a SCI-tive, both on the floor tray of SCI-tive and on a passive anti-vibration plate placed on the floor tray inside SCI-tive. The amount of vibration reduction was measured.

Materials and Equipment

	Materials	Catalogue #	Supplier
1	SCI-tive	RUS.SCI001N	Baker Ruskinn, Wales, UK
2	H9 human embryonic stem cells	HTB-176	ATCC
3	Black 96-well cell culture dishes, tissue culture treated	655090	Greiner Bio-One
4	mTESR	05850	StemCell Technologies
5	Accutase	A1110501	Life Technologies
6	Knockout-DMEM	10829-018	Life Technologies
7	Rho Kinase inhibitor Y27632 dihydrochloride	AB120129	Abcam
8	Vybrant Cell Adhesion Assay	V13181	Life Technologies
9	SpectraMax® M3 Multi-Mode Microplate Reader	M3	Molecular Devices
10	Heraeus™ Multifuge™ X1 R Benchtop Centrifuge	75004251	Thermo Scientific

Methods

To quantify cell adhesion, cells labeled with Calcein were plated and a fold-change in adherence was quantified relative to a control (adherence in an incubator). Upon entry into cells, Calcein precursors undergo cytoplasmic metabolism by endogenous esterases to produce intracellular Calcein with a highly stable, fluorescent signal comparable to that of FITC (absorbance and emission at 494 nm and 517 nm respectively). A cell adhesion assay based on entry and detection of Calcein into cells was used according to the manufacturer's instructions (Vybrant Cell Adhesion Assay, Cat # V13181, Life Technologies).

The H9 human embryonic stem cell (hESC) line was maintained in mTESR (Cat # 05850, StemCell Technologies) prior to experiments and showed morphology typical to that of pluripotent stem cells. Prior to Calcein labeling, cells were dissociated into a single cell state by 5-minute incubation with Accutase (Cat # A1110501, Life Technologies) and an inhibitor of Rho Kinase to prevent dissociation-induced apoptosis at a final concentration of 10 µm (Y27632 dihydrochloride, Cat # AB120129, Abcam). Cells were then collected in warm, serum-free medium (Knockout-DMEM, Cat # 10829-018, Life Technologies) and centrifuged at 230 x g for 3 minutes. Cells were washed twice with medium followed by repeated centrifugation to remove all traces of serum (inhibitory to Calcein uptake). The cell pellet was then aliquoted at 2.5 x 106 cells per 1ml fresh medium and incubated with 5 mM Calcein for 30 minutes at 37° C to allow cellular uptake. Three additional washes with fresh medium were then performed to remove free, unabsorbed Calcein. Labeled cells were plated either at a serial dilution for formation of a standard curve (**Figure 1**) or at 2.5 x 105 cells per well of a 96-well plate for adhesion assays (**Figure 2**). Cells were allowed to attach for 60 minutes before three washes with fresh medium to remove non-adherent cells. 100 µl of fresh media was added to each well prior to analysis using a fluorescent plate reader set for excitation at 485 nm and emission at 520 nm. Adhesion was quantified by subtracting background medium fluorescence and normalising absorbance values to adherence in a standard incubator. Data is expressed as mean ± standard error with significance set at $p < 0.05$.

Results

Initial assessment required formation of a standard curve using serial dilutions of cell numbers with a starting concentration of 5 x 106 cells down to 625 cells (**Figure 2**). Standard curve confirmed a fluorescent signal that was sensitive to alterations in cell number. 2.5 x 105 cells/well was identified as a suitable density for adhesion assays (high signal output with allowances for decreases in cell numbers to be detected). Signal was not evident at or below 5 x 103 cells / 96 well.

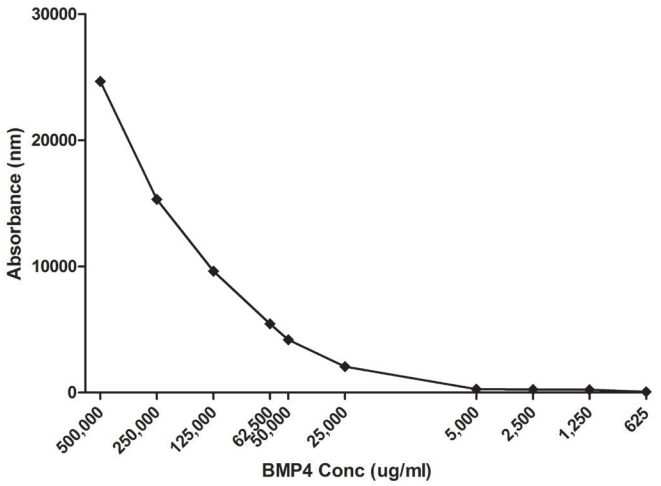

Figure 2. *Calcein labeled cells in serial dilutions show corresponding decrease in absorbance at 520 nm.*

For the initial round of experiments, cell adhesion assays as described above were performed in a standard CO_2 incubator, on the floor tray of SCI-tive and on an anti-vibration plate in SCI-tive (see **Figure 3**). When normalized to the adhesion of cells in a standard incubator, cell adhesion in SCI-tive was comparatively reduced on both the floor tray and on the anti-vibration plate (78.6 ± 1.4% and 86.8 ± 7.0% respectively) with no significant benefit on cell adhesion in SCI-tive due to use of the anti-vibration plate (p = 0.31).

The SCI-tive floor tray was then removed and ferrules ("rubber feet") placed onto the ends of the acrylic pillars before replacing the floor plate and repeating the experiment.

The addition of the ferrules significantly increased adhesion in SCI-tive to above that of a standard incubator, both with use of the floor tray and the anti-vibration plate (adhesion at 107.9 ± 2.9%, p=0.0008 and 125.27 ± 4.7%, p = 0.01 respectively, **Figure 3**).

The combination of the anti-vibration plate and the floor plate ferrules also significantly increased cell adhesion in comparison to the ferrules and the floor tray alone (p = 0.03).

Next, it was next tested whether adding more weight to the SCI-tive floor or anti-vibration plate would decrease the amount of vibration and, therefore, increase cell adhesion. Upon addition of a 5 kg weight, there was no difference in any of the conditions (**Figure 4**). Adding more weight

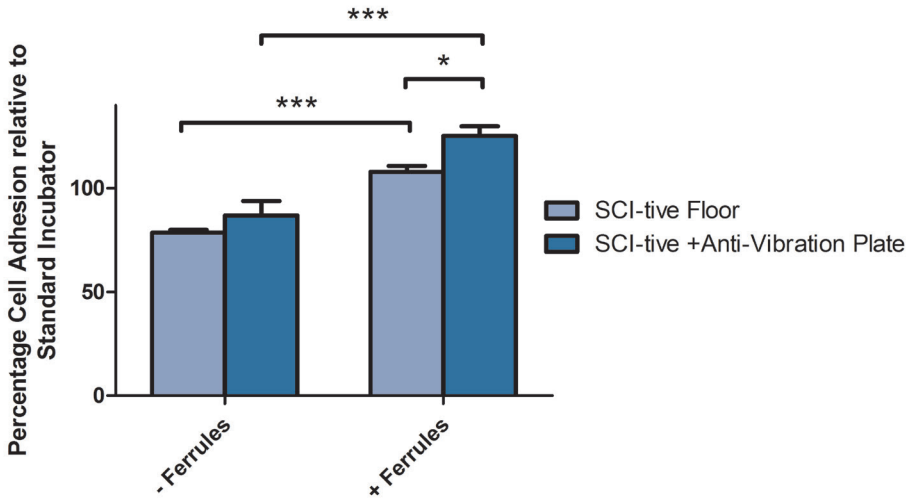

Figure 3. *Addition of ferrules augments cell adhesion in the SCI-tive. Use of ferrules significantly increased adhesion on the floor tray from 78.6 ± 1.4% to 107.9 ± 2.9%, and on the anti-vibration plate from 86.8 ± 7.0% to 125.27 ± 4.7%. p < 0.05 denoted by *, p < 0.001 denoted by ***.*

negates the benefit of adding ferrules to the SCI-tive floor and the anti-vibration plate as compared to cells grown in a standard incubator.

The amount of vibration reduction was measured using an Android Nexus 7 tablet loaded with the Physics Toolbox app. The amount of vibration was recorded for standard mounting, all 15 ferrules attached to anti-vibration plate, and 15 ferrules anti-vibration plate touching the back wall. As seen in **Table 1**, adding the 15 ferrule feet to the anti-vibration plate greatly reduces vibration in all dimensions. This is not true, however, when the plate is touching the back wall. Therefore, it is critical to be mindful of the equipment placement within SCI-tive to ensure minimal vibration effects.

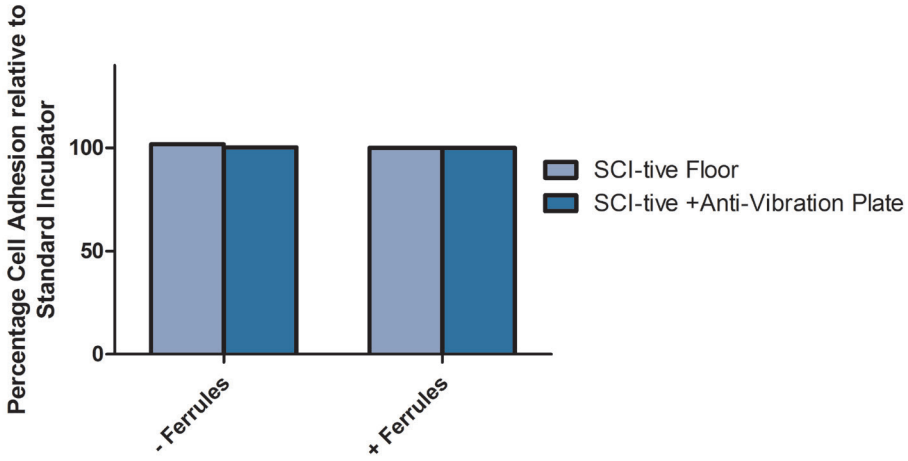

Figure 4. *Weighting of the SCI-tive floor or anti-vibration plate removes the beneficial qualities of combating vibration over a standard incubator. A 5 kg weight was added to the SCI-tive floor and anti-vibration plate and the cell adhesion assay run. Rates are shown as percentage of cell adhesion compared to a standard incubator.*

Configuration	Percent Reduction in Vibration		
	X-axis	Y-axis	Z-axis
Standard mounting	0%	0%	0%
Standard touching rear wall	-13%	-17%	20%
15 ferrule feet fitting	27%	59%	30%
15 ferrule feet touching rear wall	-53%	31%	41%

Table 1. *Vibration reduction using ferrules on the anti-vibration plate. The amount of vibration reduction was measured on the anti-vibration plate with and without ferrules and/or contact with the rear wall.*

Conclusions

Addition of the ferrules supporting the SCI-tive floor significantly increased cell adhesion, acting not only to cancel out the attenuated adhesion seen without them, but also to augment adhesion to above that of a standard incubator (**Figure 3**). This effect is likely due to a "muffling" effect of the vibrations within SCI-tive on the floor tray. Applying weight to either the SCI-tive floor or anti-vibration plate did not enhance this phenomenon, but rather negated it (**Figure 4**). This data highlights the benefits of using a SCI-tive workstation to enhance cell culture as compared to standard incubators. This would be critical for sensitive cell lines or precious cells with low numbers. Being able to rescue more cells from cultures increases productivity and helps to keep cells in their natural environment.

References

Kay, A. G., Dale, T. P., Akram, K. M., Mohan, P., Hampson, K., Maffulli, N., Spiteri, M. A., El Haj, A. J., Forsyth, N. R. (2015) BMP2 repression and optimized culture conditions promote human bone marrow-derived mesenchymal stem cell isolation. *Regenerative Medicine*. 10(2), 109–125.

To learn more about SCI-tive hypoxia workstation from Baker Ruskinn, please visit bakerco.com/sci-tive

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