Effect of laboratory lights on cell culture media

Turning out the light can be more beneficial than you think. This study focuses on the effects of laboratory light on cell culture medium. Our results show that light exposure has a deleterious effect on DMEM. Cell yields in DMEM exposed to light were 10% to 40% of those in non-exposed DMEM. Fetal bovine serum (FBS) was shown to be relatively stable to light exposure and was even shown to have a protective effect on cell culture medium against the light-induced loss of growth capacity.

Introduction

Cell cultures are often covered to protect the culture medium from ambient light during storage, routine cell maintenance, and experimental setup. But does light affect cell culture medium prior to contact with the cells? Our review of literature showed fluorescent light can have a surprisingly strong effect on the growth of mammalian cells. Evidence suggests multiple mechanisms, including production of toxic photoproducts involving riboflavin, tryptophan, or tyrosine (1, 2). Other researchers report that supplementation of these components following light exposure of complete Dulbecco's modified Eagle's medium (DMEM) does not restore the growth capacity of the medium (3). One explanation attributes the reduction in growth to the production of toxic photoproducts. Photoactivation of riboflavin leads to tryptophan-free radical production accompanied by the formation of peroxides (4, 5). In one study, the addition of catalase to light-exposed DMEM reduced loss of growth by 40%. The other 60% of reduction in growth was presumably mediated by non-peroxide photoproducts. This finding can partially be attributed to the observation that under aerobic or anaerobic conditions, a photoadduct of tryptophan and riboflavin is produced in tissue culture medium exposed to light (6).

In spite of earlier reports, the potential deleterious effects of ambient light have largely been overlooked. The phenomenon is associated with the portion of the light spectrum below 540 nm (7). You can minimize exposure to these wavelengths emanating from fluorescent lights by covering medium bottles with yellow bags and by covering fluorescent lights in storage areas and laboratories with yellow tubes.

In this study, DMEM without serum was exposed to ambient levels of laboratory light for 48 hours. Furthermore, we studied to what extent performance characteristics of FBS are affected by exposure to laboratory light and whether FBS will alter the light-associated effects on the medium.

Materials and methods

Cells and media

AIF (a murine hybridoma line producing IgG specific for alpha intermediate filament), Balb/3T3 (mouse embryo fibroblast line), and BHK-21 (baby hamster kidney fibroblast-like line) were used in these studies. DMEM was used as the basal medium. For cell culture, the DMEM was supplemented with 10% FBS. DMEM samples that contained 10% FBS during exposure were used to culture the cells without further supplementation. DMEM that contained no serum during exposure was supplemented after exposure with non-exposed FBS. For studies on effects of light on serum performance, exposed serum was added to non-exposed DMEM.

Light exposure of DMEM

We exposed DMEM in 125 mL clear plastic medium bottles to a Sylvania warm white 40 W fluorescent bulb. The samples were exposed for 48 h at room temperature (22°C) to light intensities of 105 and 160 dekalux (1 dekalux = 0.929 footcandle) by varying the distance between the medium bottles and the light source. We also placed some bottles in translucent, yellow plastic bags and exposed them to 160 dekalux. Light intensity at the surface of the medium bottles was measured using a model 408-2 illumination level meter (Simpson Electric Co., Elgin, IL). We also exposed deficient DMEM (without riboflavin, tryptophan, or tyrosine) at 160 dekalux for 48 h. Riboflavin, tryptophan, and tyrosine were added to the deficient DMEM after exposure to light. A non-exposed control medium was placed in a lightproof box. Exposed media were supplemented with 10% FBS after exposure.

Light exposure of serum and DMEM containing serum

All exposures took place at room temperature (22°C) in 125 mL clear plastic (PETG) medium bottles. The light source was a Sylvania warm white 40 W fluorescent bulb. Light intensity at the surface of the medium bottles was measured using a model 408-2 illumination level meter (Simpson Electric Co., Elgin, IL). Exposure intensity was controlled by varying the distance between medium bottles and the light source. Samples were exposed at 105 dekalux, which is the median light intensity for laboratory workbenches and tissue culture hoods (1). Non-exposed controls were identical samples shielded in a lightproof box on the exposure bench throughout the corresponding exposure period.

Cell yield studies

Cells were cultured in 24-well plates (1.9 cm^2/well), with 1 mL of medium/well. Each condition was performed in triplicate. Seeding densities were 25 000 cells/well for AIF cells, 15 000 cells/well for Balb/3T3 cells, and 7500 cells/well for BHK-21 cells. Cells were cultured at 37°C in a humidified 5% CO_2/95% air atmosphere in the dark for three to five days. At indicated times after seeding, cells were harvested and counted using a model ZM counter (Coulter Electronics, Inc., Hialeah, FL).

Cloning efficiency

BHK-21 cells were seeded into 96-well plates at a cell concentration of 5 cells/mL in control medium or previously exposed test medium (all containing 10% FBS). Each well received 0.2 mL of cell suspension. Three 96-well plates were used for each condition. The number of wells containing clones was counted 10 to 14 days after seeding. Cloning efficiency was calculated by dividing the number of wells with observed clones by the number of wells calculated to have received one or more cells based on the Poisson distribution and an average seeding density of 1 cell/well.

Results

Effect of light exposure on performance of DMEM

Exposure of cell culture medium to commonly encountered levels of fluorescent light can have major effects on the growth of cells *in vitro*. Many researchers have attributed a loss of culture growth to medium deterioration. The growth data obtained in this study are shown in Figure 1 and corresponding micrographs of cells are shown in Figure 2. Cell proliferation in medium exposed to 105 dekalux was 20% to 35% lower than that of control medium for all three cell lines. There was no reduction in growth in medium placed in a translucent yellow bag.

Previously, it was shown that a combination of riboflavin, tryptophan, and tyrosine must be present in the medium during light exposure for a similar light-mediated effect to occur with the human cell line D98/AH_2 (1, 2). We observed similar effects with the Balb/3T3 and BHK-21 cell lines, wherein the deleterious effect of light exposure was diminished in absence of these components. In two of the cell lines, Balb/3T3 and BHK-21 (C-13) cells, the growth in exposed deficient medium was only slightly depressed relative to unexposed medium. Growth was reduced by 50% in the AIF cell line. However, this still exceeded growth in complete DMEM exposed to 105 dekalux. Other components may affect growth in the AIF cell line.

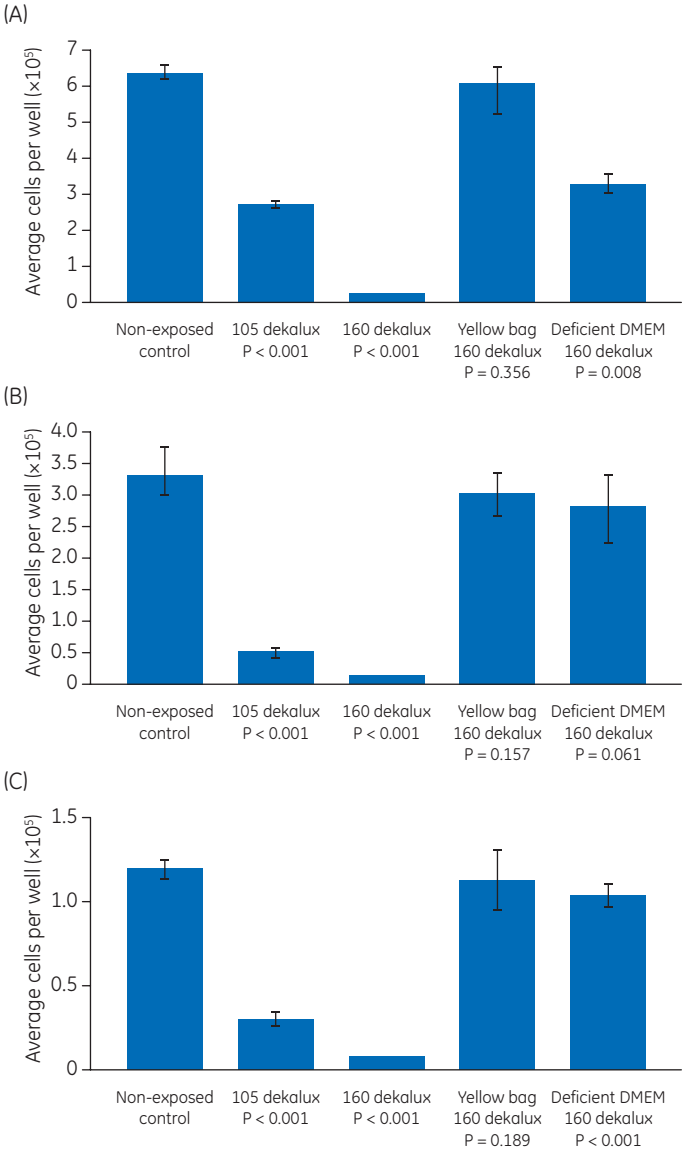


Fig 1. Effect of light exposure on culture medium. DMEM was exposed to the indicated intensities of warm fluorescent light for 48 h before used to culture cells. Cell yields were determined from triplicate counts on three cultures per condition and P values indicate comparisons with cell yields in non-exposed control medium: (A) AIF hybridoma cells, (B) BHK-21 (C-13) cells, and (C) BALB/3T3 cells. Deficient DMEM = without riboflavin, tryptophan, or tyrosine.

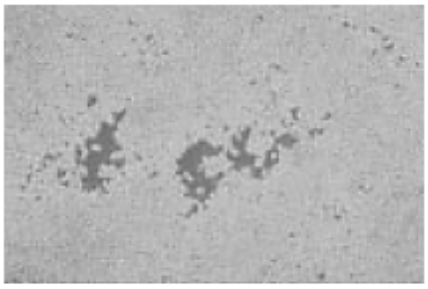
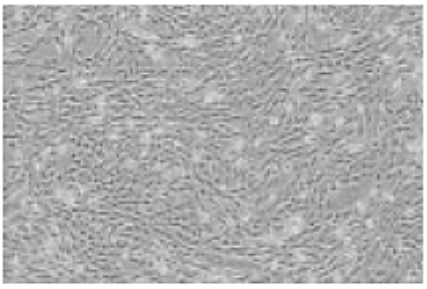
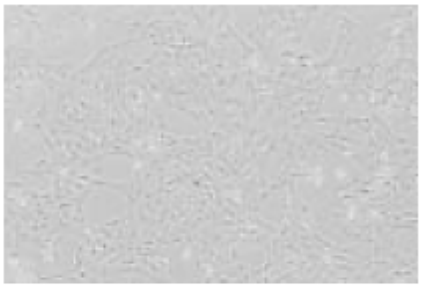
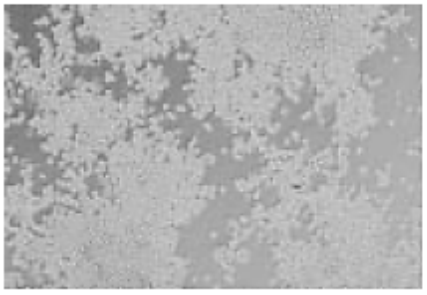
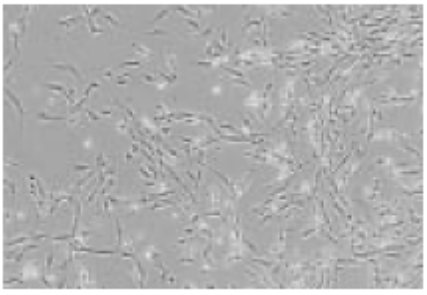
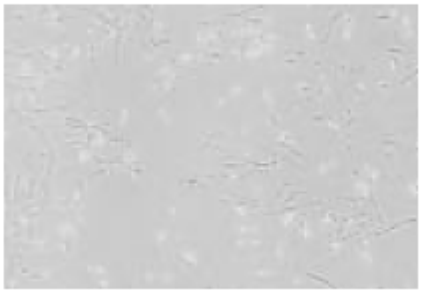
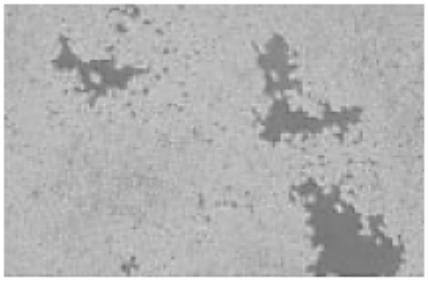
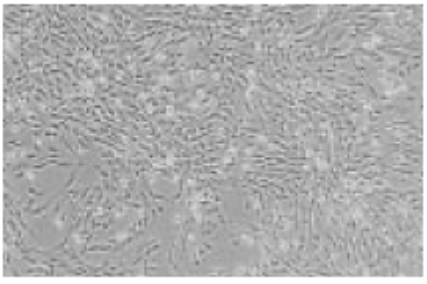
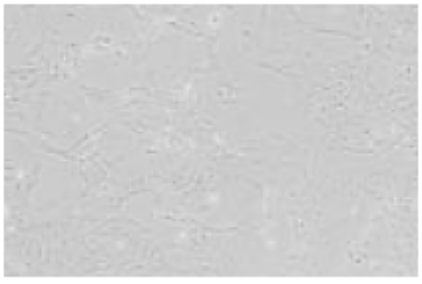
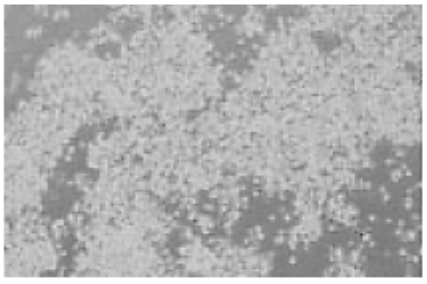
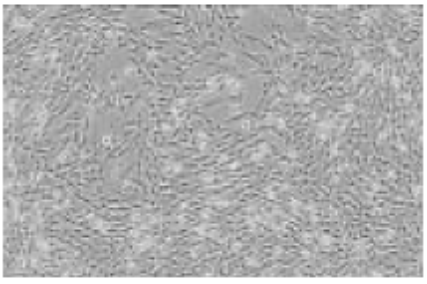
AIF	BHK-21	BALB/3T3
		
AIF non-exposed control	BHK-21 non-exposed control	BALB/3T3 non-exposed control
		
AIF exposed 105 dekalux	BHK-21 exposed 105 dekalux	BALB/3T3 exposed 105 dekalux
		
AIF exposed 160 dekalux	BHK-21 exposed 160 dekalux	BALB/3T3 exposed 160 dekalux
		
AIF exposed covered with yellow bag 160 dekalux	BHK-21 exposed covered with yellow bag 160 dekalux	BALB/3T3 exposed covered with yellow bag 160 dekalux
		
AIF exposed deficient DMEM (without riboflavin, tryptophan, or tyrosine) 160 dekalux	BHK-21 exposed deficient DMEM (without riboflavin, tryptophan, or tyrosine) 160 dekalux	BALB/3T3 exposed deficient DMEM (without riboflavin, tryptophan, or tyrosine) 160 dekalux

Fig 2. Growth of three cell lines in medium exposed to laboratory light. Micrographs of cultures correspond to data in Figure 1.

Effect of light exposure on performance of FBS

When FBS was exposed at 105 dekalux for 24 to 48 h before evaluated in cell yield assays, exposed and non-exposed control FBS appeared equivalent. The length of exposure was increased to examine the limits of this relative stability. Following 21 days of exposure at 105 dekalux, the performance of FBS as measured by cell yield assays was similar to that of non-exposed FBS with the 3T3 and BHK-21 cells and slightly reduced with AIF cells (Fig 3).

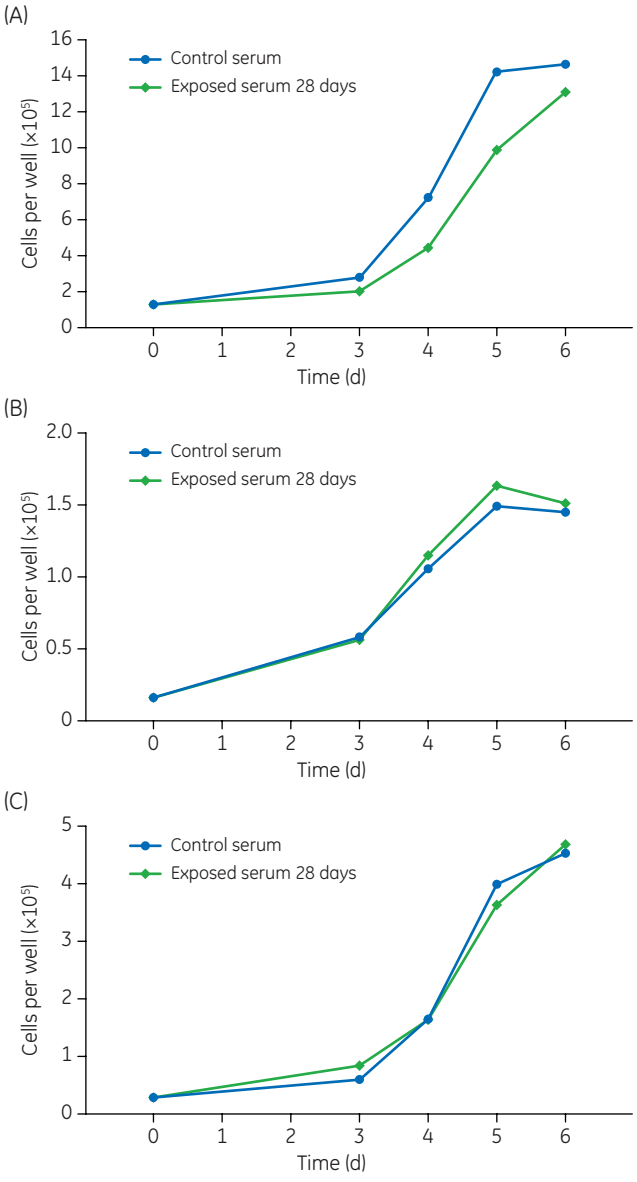


Fig 3. Effect of light on FBS performance. Cell yields for cells cultured in unexposed DMEM supplemented to 10% with FBS that had been stored for 21 days either at 22°C in dark (control serum) or at 22°C in light at 105 dekalux (exposed serum). (A) AIF hybridoma cells, (B) BALB/3T3 cells, and (C) BHK-21 (C-13) cells.

Cell yield assays did not show a significant decline in performance of light-exposed FBS. Cloning efficiency, an inherently more sensitive assay, was used to compare cell culture performance of exposed and non-exposed FBS. Cloning efficiencies in FBS that had been exposed for 28 days at 105 dekalux revealed a decline in cell culture performance (Fig 4). In unexposed DMEM supplemented to 10% FBS with the exposed FBS, the cloning efficiency of BHK-21 cells was 17% ± 3% compared with 30% ± 3% in medium supplemented with the unexposed control FBS (Fig 4). This 43% relative decline ($P = 0.008$) in cloning efficiency was attributed to light-dependent mechanisms as the unexposed control FBS had sat at room temperature in a light-proof box for 28 days beside the FBS being exposed to light. During the 28 days at room temperature, there was a decline ($P < 0.001$) in cloning efficiency with the non-exposed control FBS as compared with the cloning efficiency of 47% ± 5% in FBS that had been stored at 4°C in the dark during the 28 days (Fig 4).

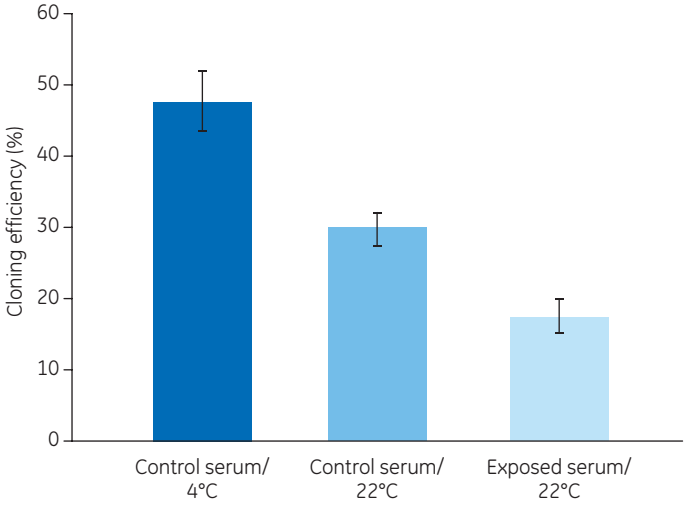


Fig 4. Effect of light and temperature on FBS performance. Cloning efficiency for BHK-21 cells in unexposed DMEM supplemented to 10% with FBS that had been stored for 28 days either at 4°C in dark (control serum/4°), at 22°C in dark (control serum/22°), or at 22°C in light at 105 dekalux (exposed serum/22°).

Does FBS present in basal medium during light exposure alter the effects of light exposure on cell culture performance?

DMEM either with or without 10% FBS was exposed for 48 hours at 105 dekalux. At the end of the exposure period, the DMEM/10% FBS control and DMEM/10% FBS exposed medium were used to culture cells without further supplementation, while the exposed serum-free DMEM and control medium were supplemented with unexposed FBS before evaluated. The results of cell yield assays show that with all three cell lines, the cell culture performance of medium declines following light exposure (for all control vs exposed, cell yield comparisons, $P < 0.01$) (Fig 5).

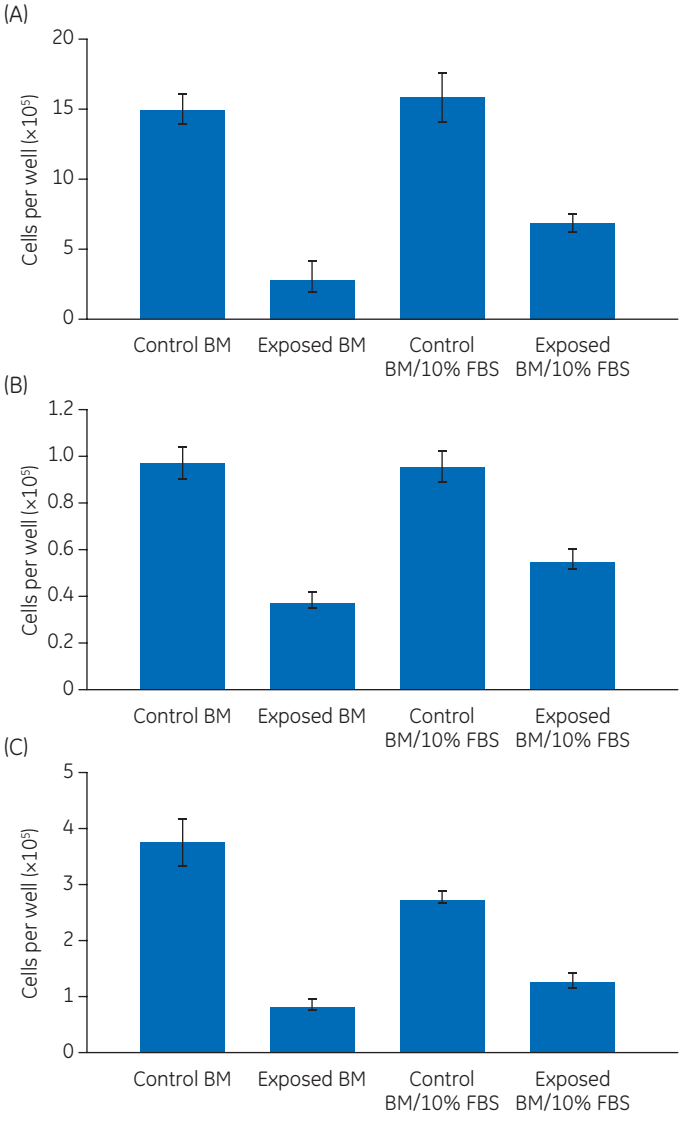


Fig 5. Effect of light on basal medium (BM) with and without FBS. Non-supplemented BM and BM containing 10% FBS (BM/10%FBS) were stored at 22°C in dark (control) or exposed at 22°C to light at 105 dekalux for 48 h. Following exposure, the non-supplemented BM was supplemented with non-exposed FBS. Cell yields were determined after 5 days in these media. (A) AIF hybridoma cells, (B) BALB/3T3 cells, and (C) BHK-21 (C-13) cells.

For medium exposed without FBS and with FBS, cell yields for BHK-21 cells declined 76% and 54%, respectively. As a measure of culture medium performance, cloning efficiency is more sensitive than cell yield. Even after a shorter exposure period, 24 h rather than 48 h, the cloning efficiency for BHK-21 cells dropped 96% and 90% in serum-free DMEM and DMEM/10% FBS, respectively (Fig 6).

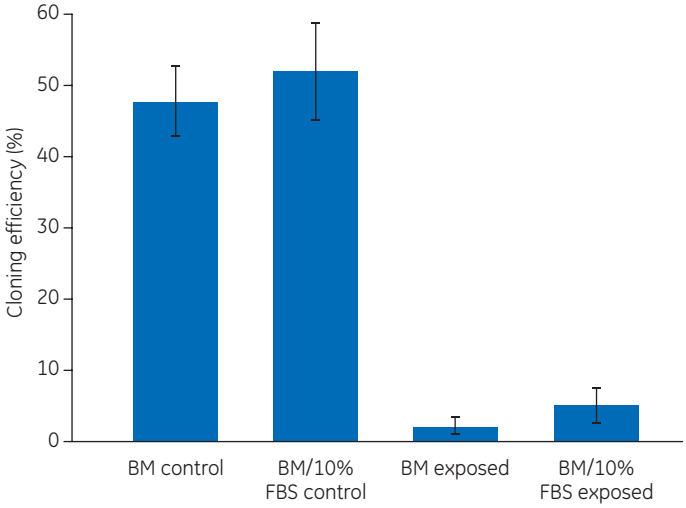


Fig 6. Effect of prior light exposure on the ability of basal medium (BM) to support cloning of BHK-21 cells. BM containing either no FBS or 10% FBS was exposed at 22°C for 24 h at 105 dekalux, controls were at same temperature, but shielded from light. Following exposure, the non-supplemented BM was supplemented with non-exposed FBS.

Conclusion

The sharp decline in growth-supporting capacity of DMEM exposed to fluorescent light has been attributed to two mechanisms: the photoactivation of riboflavin leading to tryptophan free radical production accompanied by peroxide formation (5, 6) and the formation of photoadducts of riboflavin and tryptophan (7). Our results show that cell yields in DMEM that had been exposed to light were 10% to 40% of those in non-exposed DMEM. The loss of growth capacity was dose-dependent, with greater declines observed with either higher light intensities or longer exposure periods.

Furthermore, we examined the effect of exposure to laboratory light on FBS. The data indicate that FBS as compared with DMEM is relatively stable to light exposure and partially stabilizes cell culture medium against the light-induced loss of growth capacity. Some of the protective effect of serum can be attributed to the catalase activity in serum (5), which could interrupt free radical-mediated reactions. Both light exposure and prolonged storage at room temperature resulted in declines in cell culture performance of FBS when performance was measured by cloning efficiency. In fact, the decline attributable to exposure to light for 28 days was nearly equal to the decline attributable to storage at room temperature (22°C) rather than at refrigerator temperature (4°C) for 28 days. Both conditions resulted in approximately 40% reductions in relative cloning efficiency.

As demonstrated here, the deleterious effects of laboratory lights on cell culture medium performance can be reduced by keeping medium in the dark or in protective yellow bags. Further precautions may include covering fluorescent lights in storage areas and cell culture hoods with yellow plastic films. These same procedures are recommended as ways to limit the harmful effects of light on serum where serum can be exposed for an extended time or to repeat short exposures.

References

1. Nixon, B.T. and Wang, R.J. Formation of photoproducts lethal for human cells in culture by daylight fluorescent light and bilirubin light. *Photochemistry and Photobiology* **26**, 589-593 (1977).
2. Wang, R.J. Effect of room fluorescent light on the deterioration of tissue culture medium. *In Vitro* **12**, 19-22 (1976).
3. Stoien, J. D. and Wang, R.D. Effect of near-ultraviolet and visible light on mammalian cells in culture II: Formation of toxic photoproducts in tissue culture medium by black light. *Proc Nat Acad Sci* **71**, 3961-3965 (1974).
4. Sanford, K. K., Parshad, R., and Gantt, R. Responses of human cells in culture to hydrogen peroxide and related free radicals generated by visible light: Relationship to cancer susceptibility. In Free radicals, aging and degenerative diseases. Wiley-Liss, Inc, New York pp. 373-394 (1986).
5. Wang, R.J. and Nixon, B.T. Identification of hydrogen peroxide as a photoproduct toxic to human cells in tissue-culture medium irradiated with "daylight" fluorescent light. *In Vitro* **14**, 715-722 (1978).
6. Silva, E., Salim-Hanna, M., Becker, M.I., and De Ioannes, A. Toxic effect of a photoinduced tryptophan-riboflavin adduct on F9 teratocarcinoma cells and preimplantation mouse embryos. Internat. *J Vit Nutr Res* **58**, 394- 401 (1988).
7. Wang, R.J. Lethal effect of "daylight" fluorescent light on human cells in tissue-culture medium. *Photochemistry and Photobiology* **21**, 373-375 (1975).

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