Examining the Relationship Between Media and Light

by William H. Siegel

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Introduction

Most tissue culturists will never observe effects on proliferating cells that can be attributed to light-exposed media. However, several light-related factors may exert sufficient collective influence to cause the spurious results occasionally encountered in critical media applications. This tutorial is designed to increase awareness that tissue culture media may be photosensitive and to improve media handling so that any possible adverse effects are minimized.

Tissue culture media have been reported to react with exposure to light, yielding photoproducts that may adversely affect cells cultured in a given medium (1-6). The effects are mediated by riboflavin, also known as vitamin B2 (1,7), and the essential amino acid tryptophan (8-11), although tyrosine may play a lesser role (2,3,5,9,12).

Mechanisms

Adverse effects of light exposure on cells in culture are attributable to riboflavin-related production of free radicals followed by generation of peroxides and other photoproducts. Photoeffects disappear when riboflavin is deleted from the media. Hydrogen peroxide (H_2O_2) is produced by free radical reactions and accounts for most, but not all, observed cytotoxicity (2,6,7,13-15). The effects, if any, of light exposure on tissue culture media depend on several variables.

Light source. The visible light wavelength spectrum is 400-700 nm for violet to red light. Ultraviolet (UV) wavelengths are below 400 nm. Photoeffects are observed at wavelengths below 540 nm (4). Common laboratory fluorescent lamps emit significant radiation in that range, as do tungsten sources and sunlight. Riboflavin absorbs light maximally at 375 and 447 nm (5). Wavelengths below 300 nm are blocked by culture vessels (3). Of the wide variety of fluorescent tubes available, some have emission spikes at wavelengths near riboflavin's maximal absorbance. A greater photoeffect could be expected from such tubes.

Total light dose. Some investigators have reported light intensities and dosages in units that are not meaningful to most laboratory workers. For the purposes of this tutorial, I converted all light intensity units to watts per square meter (W/m^2). I also compared the figures with typical laboratory light intensity of 1-2 W/m^2 or an average of 1.5 W/m^2 (4). Conversion figures are based on data from the CRC Handbook of Chemistry and Physics (16). Total light doses are expressed here as Intensity X Time in watt-hours per square meter ($W\text{-}H/m^2$).

Photoeffects depend on the total dose of light absorbed by a culture medium. Repeated exposure of medium- to low-intensity light was consistently found to stimulate proliferation of human diploid cells, whereas high intensity and/or prolonged exposure was growth-inhibitory or even cytotoxic (17). For critical media applications (such as cloning efficiency), cytotoxic effects may be observed at a total dose of 10-15 $W\text{-}H/m^2$ (4,6,7,9). That is equivalent to exposure at a typical laboratory light intensity of 1.5 W/m^2 for 6-10 hours.

HEPES effects. HEPES buffer is reported to cause increased photosensitivity of tissue culture media. One study showed that with a light intensity of 2 mW/cm^2 and a total exposure of 60 $W\text{-}H/m^2$ (40 hours at typical laboratory light intensity), RPMI supplemented with 25 mM HEPES almost totally inhibited cell proliferation (7). Proliferation was reduced by about 25% after an exposure equivalent to 6.6 hours of typical laboratory light. The same study demonstrated that an identical light dose (60 $W\text{-}H/m^2$) had no significant adverse effect in the absence of HEPES.

Spierenburg revealed that light-exposed HEPES-buffered RPMI inhibited cellular proliferation in direct proportion to the HEPES concentration (18). Spierenburg's light unit conversion figure is inconsistent with data (1 lux-hr = 5.29 $Joule/m^2$) found in the CRC Handbook of Chemistry and Physics (16), which calls into question the actual light doses used to generate the published results.

Both HEPES studies cited were done with T and B cells (human and murine) that may be more photosensitive than other cell types.

Cell line sensitivity. Light-induced media cytotoxicity is a general phenomenon, but different cell lines have different tolerances to light-exposed media. For example, the human B-cell, RPMI 1788, required a sixfold higher dose of light than the human T-cell, Molt-4, to exhibit the same cytotoxic medium effects (18).

Riboflavin concentration. Riboflavin concentration directly correlates to adverse effects of light

exposure (14). Removal of riboflavin from the culture medium before exposure eliminates those effects (2,3). Stoien's results suggested that media with reduced levels of riboflavin, tryptophan, and tyrosine were likely to exhibit less cytotoxicity (3). Lucius, studying neuronal cells, found that doubled riboflavin levels produced dose-dependent increased axonal cell degeneration (1).

The riboflavin concentrations in commonly used media MEM, RPMI, and DMEM are 0.1, 0.2, and 0.4 mg/L, respectively. In contrast, Medium 199 and Ham's F12 have riboflavin levels tenfold lower (0.01 and 0.04 mg/L, respectively) and are less photosensitive. Table 1 shows the concentrations of various media components linked to photosensitivity.

Media (classic formula)						
Component	**MEM**	**DMEM**	**RPMI 1640**	**Medium 199**	**F12**	**BME**
Ascorbic acid	0.0	0.0	0.0	0.05	0.0	0.0
Glutathione, reduced	0.0	0.0	1.0	0.05	0.0	0.0
HEPES	0.0	0.0	0.0	0.0	0.0	0.0
Pyruvate	0.0	110.0	0.0	0.0	110.0	0.0
Riboflavin	0.1	0.4	0.2	0.01	0.04	0.04
L-Tryptophan	10.2	16.0	5.0	10.00	2.04	4.00
L-Tyrosine	36.2	72.0	20.0	40.00	5.44	18.10
Vitamin E	0.0	0.0	0.0	0.01	0.0	0.0

Table 1. Concentrations (mg/L) of media components linked to photoeffects.

Cell density. Light-mediated effects depend on cell density (14). No cytotoxic effect was observed at cell densities significantly higher than clonal densities (2). Although cloning efficiency may be the most sensitive method of evaluating culture medium performance, much tissue culture work is conducted at higher than clonal (low) cell densities.

Oxygen concentration. Medium that has been depleted of dissolved oxygen is nearly devoid of photoreactivity (7). Bioreactor systems with elevated oxygen levels may have increased sensitivity to light.

Population doubling level. WI-38 human diploid lung cells that have doubled in number few times and that have a low population-doubling level (PDL) are reported to be more sensitive to light exposure than cells at higher PDLs (14).

Prevention

Riboflavin avoidance. To decrease photoreactivity, consider using an isotonic salts solution (such as Hank's balanced salts solution) instead of a riboflavin-containing medium whenever possible.

Antioxidants. Photoeffects are eliminated by antioxidants. Parshad demonstrated that light-induced chromatid breakage was not significantly different than that for the unexposed control when ascorbic acid (0.1 mg/mL) was added to the medium together with reduced glutathione (0.02 mg/mL) (13). Others have shown that ascorbic acid (1-5 µM), vitamin E (1-5 µM), and pyruvate (2-10 mM) prevent adverse effects (1,18).

The reported increase in photosensitivity of media containing HEPES may be due to a lack of antioxidants. Studies cited here showing increased sensitivity with HEPES used either RPMI or BME medium. The standard formulation BME medium lacks antioxidants, and RPMI contains only reduced glutathione at a 20-fold lower level than was reported effective above. Other studies cited here used classically formulated DMEM containing 1 mM pyruvate, which is likely to mitigate some photoeffects.

Wavelength selection. Tinted plastic sleeves that slide over fluorescent tubes are available at low cost and ensure the absence of photoeffects on media. Such filtration sleeves, transmitting in the range of 575-700 nm, are a better choice than fluorescent tubes that emit colored light, which can leak light at the tube ends. Defects in the colored lamp phosphor may cause emission of harmful wavelengths (4,9,14). "Sterilizing" UV lamps commonly found in tissue culture hoods should not be used when working with media or cells inside the hood. Short and ultraviolet wavelengths are most injurious to media and cells.

Dark storage. Dark storage of light-struck media at 4 °C for 2-3 weeks eliminates cytotoxic or inhibitory effects previously present (15,19). Cell proliferation using light-irradiated medium previously stored 18 days in the dark at 4 °C was equivalent to that for the control.

Media manufacturers use clear bottles to permit observance of media color (pH) and to confirm that media are free of contamination or precipitate. Shipping cartons shield the bottles from light exposure. Protect media that can be stored at room temperature from light. Do not store media in refrigerators with glass doors or near the light source in a cold room.

Although it is possible for light-exposed tissue culture media to cause adverse cellular effects, such effects are unlikely to be observed in the majority of media applications. Extended exposure time is required to elicit adverse effects at typical light intensities (1-2 W/m^2). Several measures can be employed to prevent or mitigate such photoeffects in situations that may combine several risk factors. Therefore, although it should be considered in production and experimental planning, light sensitivity of media need not be an overriding concern.

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William H. Siegel is a senior technical service representative for Cambrex Bio Science Walkersville, Inc., 8830 Biggs Ford Road, Walkersville, MD 21793, (301) 898-7025, fax (301) 845-2924, william.siegel@cambrex.com.

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