European Cells and Materials Vol. 10. Suppl. 5, 2005 (page CS1) ISSN 1473-2262

Engineering Interfaces to Cells using Synthetic Liquid Crystals

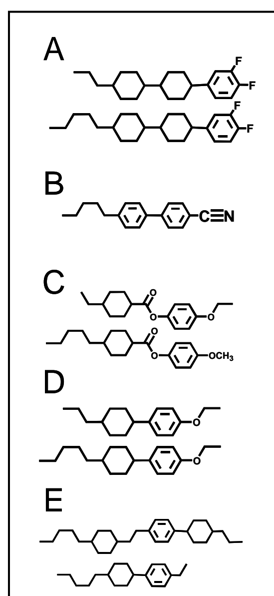
Nicholas L. Abbott, Nathan Lockwood, Christopher J. Murphy, Li-Lin Cheng, Chang-Hyun Jang

Dept of Chemical & Biological Engineering, University of Wisconsin-Madison, Madison, Wisconsin, 53706, USA

INTRODUCTION: Given the ubiquitous presence of the liquid crystalline state in biological systems and the technological utility of liquid crystals, it is surprising that few examples of the use of liquid crystal technologies involving whole mammalian cell have been reported. One could envisage, for example, the use of liquid crystals to image the expression and organization of receptors either expressed on the surfaces of cells (or secreted into the liquid crystal) in response to biochemical or biophysical cues. Alternatively, liquid crystals might be exploited to deliver chemical or mechanical stimuli to cells and thereby guide their behavior.

To enable the development of technologies that interface cells with liquid crystalline environments, liquid crystals that are not toxic to cells are required. We report here the results of an initial search for liquid crystals that are *not* toxic to mammalian cells.[1] In this work, we carried out experiments using living cells immersed under eight thermotropic liquid crystal mixtures to screen for chemical functionalities in liquid crystals that maintain the viability of cells. Each liquid crystal mixture was comprised of mesogens that contained a unique set of functional groups.

METHODS: TL205, 5CB and E7 were purchased from EM Industries (Merck), NY. Components for the cholesteric series were purchased from Pressure Chemical Company, PA. Components for the "A", "B", "C" and "E" series were purchased from Phentex Corporation, TX.



3T3 fibroblasts were cultured in DMEM (supplemented with 10% fetal bovine serum plus 40 μg/ml gentamycin) and grown in a humidified incubator at 37°C and 5% CO_2. SV-40 HCECs were cultured in supplemented hormonal epithelial medium (SHEM) – a basal medium for epithelial cell growth – with 10% fetal bovine serum (FBS). SHEM is a mixture of DMEM and Ham's F-12 (50/50) plus 0.5% DMSO and 40 μg/ml gentamycin. Both cell lines (3T3 fibroblasts and SV-40 HCEC) were plated at a concentration of 10,000 cells/well on a 96-well plate and allowed to attach and proliferate overnight under each cell lines' corresponding medium. The medium was then removed, 25 μl/well of either liquid crystal, cell culture medium or PBS (pH 7.4) were added to six wells for each solution (total of 120 wells). PBS was used as a control. Saponin (0.1% w/v) was added to one row of cells that had not been exposed to liquid crystal as a control for complete cell death. The cells were incubated at 37°C for 4 or 24 hours under these solutions. The solutions (liquid crystals, cell culture medium and PBS) were then removed. The cells were rinsed three times with PBS. 50 μl of ethidium-homodimer (4 μM) or 50 μl of Calcein-AM (0.6 μM) were added to each well and the plate was incubated for an additional 2 hours. Fluorescence was measured using a Cytofluor 4000TC automated fluorescent plate reader. For ethidium homodimer, the fluorescence was measured using 530 nm (excitiation) and 620 nm (emission). For Calcein-AM, fluorescence was measured using 485 nm (emission) and 530 nm (excitation).

RESULTS: In order to quantify the viability of cells treated with liquid crystals, we carried out a fluorescent assay based on intracellular esterase activity. This viability assay uses the fluorescent precursor calcein acetoxymethylester (CAL-AM), which is permeable to the membrane of cells. The presence of green fluorescence from CAL in cells is evidence of esterase activity as well as an intact membrane that retains the esterase products in the cells, both of which are indicators of a living cell.

By measuring the level of intensity of CAL fluorescence in cells treated with different liquid crystals relative to that measured without treatment with liquid crystal (in culture medium), we quantified the effect of eight liquid crystals on the viability of 3T3 fibroblast and SV-40 HCEC cells (Figure 1)

Inspection of Figure 1 reveals that, in general, the 3T3 fibroblasts and SV-40 HCECs respond in a similar manner to each liquid crystal. In particular, the "E", "A", "B" series of liquid crystals as well as 5CB and E7 are toxic to both 3T3 fibroblasts and SV-40 HCECs. The treatment of cells with these five liquid crystals caused the CAL fluorescence to decrease to less than 30% for 3T3 fibroblasts, and to less than 50% for CV-40 HCECs. In addition, it is evident that the CAL fluorescence reveals the cholesteric series of liquid crystals to be toxic to SV-40 HCECs but not 3T3 fibroblasts. However, treatment of both cell lines with the "C" series and TL205 led to levels of CAL fluorescence that were similar to the CAL fluorescence measured with cells not treated with liquid crystals (both culture medium and pure PBS buffer). We believe these two liquid crystals are not toxic to 3T3 fibroblasts and SV-40 HCECs under the experimental conditions reported in this paper.

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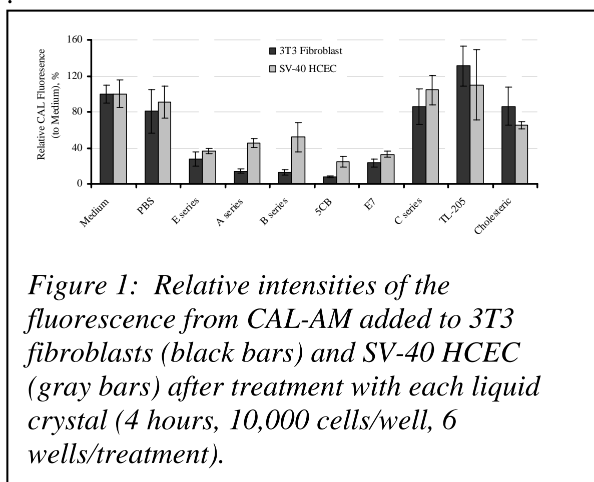

Figure 1: Relative intensities of the fluorescence from CAL-AM added to 3T3 fibroblasts (black bars) and SV-40 HCEC (gray bars) after treatment with each liquid crystal (4 hours, 10,000 cells/well, 6 wells/treatment).

CONCLUSIONS: By investigating the effects of eight liquid crystals with unique sets of functional groups on the viability of two mammalian cell lines immersed in the liquid crystals, we find that the chemical functionality of the liquid crystals correlates closely with the toxic effect (Figure 1). We identified several functional groups that, when incorporated in mesogens, were *not* toxic to cells. Treatment of cells with the liquid crystals containing these functional groups does not affect the post-treatment proliferation of the cells as compared to PBS. The mechanisms by which these functional groups define liquid crystal-cell interactions and thus toxicity are not fully understood and are the subject of ongoing research.

REFERENCES: [1] Luk, Yan-Yeung; Campbell, Sean; Abbott, Nicholas; Murphy, Christopher. "Non-toxic Thermotropic Liquid Crystals for use with Mammalian Cells." *Liquid Crystals* (2004), 31(5), 611-621.