

Assessment of the Chemical and Morphological Changes Induced in Cells by Boric Acid treatment using High Resolution Infrared Imaging

Abstract

Provide a meaningful abstract of the proposed research below. This section may be used for funding agency reporting purposes; this information and the proposal title may become public information. (limit 1300 characters including spaces)

Boric acid is found in everyday foods and drinking water in small quantities. Recent reports have suggested a link between cancer incidence and low levels of boric acid, but this phenomenon is not well characterized. In this study, we will examine the effects of boric acid on human skin melanoma cells using synchrotron FTIR imaging (FTIRI) with a Focal Plane Array (FPA) detector and Point Spread Function (PSF) image deconvolution. Cells (SK-MEL28) will be grown under concentrations of boric acid ranging from 0 to 50 mM. After 1, 2, 3, 5, 7 and 10 days, cell proliferation and survival will be determined with conventional trypan blue staining. FTIRI measurements will be performed with the new FPA detector at beamline U10B. With both synchrotron-based FTIRI and image deconvolution, we aim to image hundreds of cells rapidly at a spatial resolution of 1-2 microns. The lipid and protein maps will provide insight into cellular changes induced by boric acid, including size and shape alterations of the nuclear envelope, the distribution of protein in the cell, and the change in relative concentration of lipid vs. protein in the cytoplasm. These findings may provide details of the cellular function of boric acid in human cells and set new guidelines for the daily intake of boric acid.

Scientific Importance of this experiment

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The dietary supplement boric acid is found in everyday foods and drinking water in small quantities, but its effect on humans is not well understood. Several reports suggest a link between cancer incidence and low levels of boric acid in daily dietary consumption. This phenomenon has been observed in the case of prostate cancer and lung cancer, where an increased dietary boron intake was associated with a decreased risk of developing a cancer. A better understanding of the effects of boric acid on the proliferation and morphological behavior of human cells may provide insight into the benefit of boric acid for human health and its relation to cancer risk.

This project will investigate the effects of varying concentrations of boric acid on the proliferation of the human skin cancer cell line SK-MEL28. Concentrations will range from pharmacologically relevant to known toxic levels. FTIR imaging (FTIRI) with the new Bruker Focal Plane Array (FPA) microscope at beamline U10B will be used in order to image a large number of cells rapidly. Since the FPA is not confocal, a loss of spatial is expected. Thus, we have written a MATLAB routine to perform Point Spread Function (PSF) image deconvolution. The program, which is based on the Lucy-Richardson deconvolution algorithm, is ideal for regaining resolution lost. This is

especially useful when performing cellular imaging, as it aids in the resolution of sub-micron scale subcellular features. Here, the lipid and protein maps will provide insight into cellular changes induced by boric acid, including size and shape alterations of the nuclear envelope, the distribution of protein in the cell, and the change in relative concentration of lipid vs. protein in the cytoplasm. With this information, it can be determined how the chemistry and morphology of these regions change over time under the various concentrations of boric acid.

Why is Synchrotron Radiation required

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The cells that are to be imaged have a diameter in the range of 5 to 50 μm . The imaging of such cells with a global light source yields poor quality spectra and poor spatial resolution. Subcellular features are difficult to resolve using a global. Synchrotron radiation provides the necessary intensity to obtain high quality spectra and resolution to distinguish subcellular features.

With FTIR microspectroscopy and a single point detector, acquiring a map of a single cell requires raster-scanning to create a 2-dimensional image. For a cell with a 50 μm diameter, and using a 2 μm step size, 625 spectra are collected, which can take 12 hours to collect such a map. Alternatively, a Focal Plane Array (FPA) can be used to collect an array of spectra at once, thereby reducing the acquisition time to less than 15 minutes. However, the FPA introduced a loss in image quality due to the non-confocal nature of the FPA.

Synchrotron radiation, coupled to an FPA, greatly improves the image quality by providing a brighter light source and thus higher overall illumination of the FPA optics. The synchrotron provides higher overall illumination and a higher signal-to-noise ratio than does a global source. The higher illumination and signal-to-noise combined allow for the resolution of spectral features that are otherwise not resolvable using a global light source.

Additionally, PSF deconvolution can be applied to recover some of the resolution lost due to the non-confocal illumination as to approach the image quality delivered by conventional confocal point source microspectrometers. However, the input data must have a high signal-to-noise ratio since deconvolution algorithms inherently amplify noise. Only with high signal-to-noise data can PSF deconvolution yield high quality results.

Describe work previously performed by Principal Investigator at a synchrotron facility

(limit 2000 characters including spaces)

Alvin Acerbo is a M.S. student at Stony Brook University and has been working with Dr. Lisa Miller and Dr. Larry Carr on developing PSF deconvolution of infrared images. He has experience with the new Bruker Hyperion 3000 FTIR microscope and has imaged individual cells with the confocal FTIR microscope at U10B. The Principal Investigator for this project is Dr. Lisa Miller. She is the spokesperson for beamline U10B and has over 10 years of experience in FTIR microspectroscopy and imaging.

List up to three publications you feel will assist in reviewing this proposal:

(limit 2000 characters including spaces)

- 1) Barranco W.T., Eckhert C.D. (2006) Cellular changes in boric acid-treated DU-145 prostate cancer cells. *British Journal of Cancer* 94: 884-890
- 2) Meacham, S.L., Elwell, K.E., et al (2007) Boric Acid Inhibits Cell Growth in Breast and Prostate Cancer Cell Lines. *Advances in Plant and Animal Boron Nutrition* 299-306
- 3) G.L. Carr, O. Chubar and P. Dumas. in (Bhargava, R. and Levin, I.W., eds.) *Spectrochemical Analysis Using Infrared Multichannel Detectors*, Blackwell Publishing 2006, pp. 56-84.

Research Description

Provide sufficient details about your program to justify your request for beam time or facility time. Include a complete description of the experiments proposed over the next 2 year period (6 cycles). At the bottom of the page indicate your intention to attach a small image file with any supporting figures or diagrams. Please note that a description of your proposed plans and accomplishments for the first cycle (four month period) will be requested in another section. (4000 characters including spaces)

Cell culture.

Cell cultures will be prepared by incubating SK-MEL28 cells at 37 °C in Minimum Efficient Medium Eagle, supplemented with 10% FBS, 10% PBS (pH buffer), penicillin/streptomycin (antibiotic), and amphotericin β (antifungal). After reaching confluence, the cells will be plated into five 6-well plates containing the boric acid (B(OH)₃) rich medium and one 6-well plate without boric acid (control). Boric acid will be introduced at concentrations of 0, 5, 12.5, 25 and 50 mM. The wells of each 6-well plate are numbered 1 through 6, corresponding to the six time points (1, 2, 3, 5, 7, and 9 days). Additionally, all cells in the 6-well plates will also be grown directly onto small pieces of low-E microscope slides for direct imaging using synchrotron FTIRI.

Cell viability test.

At each time point, all cells in a well will be trypsinized with 2 mL trypsin after which the well will be mildly swirled for 30 seconds. The content of the well will be centrifuged for 10 minutes at 1000 rpm. The supernatant will be collected and set aside, and the pellet will be resuspended in 500 μ L PBS. A 200 μ L aliquot is to be used to cytopsin the cells

onto a low-E microscope slide for a duration of 4 minutes at 1000 rpm. A 10 μL aliquot will be mixed with 10 μL trypan blue, a viability indicator. 10 μL of this mixture will be loaded into each of the two counting areas of a Bright-Line hemacytometer. Living and dead cells will be counted in four 1 mm^2 areas with the use of a 200x light microscope. Living and dead cell counts will be used to calculate the number of cells per μL and the cell survival percentage at each time point for each concentration of boric acid. The supernatant collected from each well will be analyzed for its pH. These pH values and cell counts will be used to calculate the pH shift per cell due to the boric acid treatment.

FTIRI data collection.

Synchrotron FTIRI will be done using the Bruker Hyperion 3000 FTIR microscope at beamline U10B in reflection mode. The 15x visible light objective will be used to locate cells for imaging, and the 36x objective will be used to perform whole cell spectral data collection. An integration time of approximately 0.005 ms will be used for the FPA detector to prevent flooding of individual pixels, while allowing the least illuminated pixels to reach detectable readout levels. A blank low-E reflection microscope slide will be used to collect a background spectrum. To encompass all spectral features including the lipid, protein, and nucleic acid regions, the wavelength range is to be set at 3900 cm^{-1} to 850 cm^{-1} with a spectral resolution of 8 cm^{-1} . The sampling frequency will be set at four times the Nyquist frequency, and as such a 3900 cm^{-1} cutoff filter will be used to prevent fold over of high frequency features.

FTIRI data analysis.

The OPUS software program will be used to integrate the data at the lipid, amide I and amide II region in the spectra. The spectral locations of these biological components are approximately $2850 - 2950\text{ cm}^{-1}$, $1625 - 1675\text{ cm}^{-1}$, and $1525 - 1575\text{ cm}^{-1}$ for the lipid, amide I, and amide II components, respectively. Together with the visible image of the cell, the data will be exported to data files, and read in by a custom MATLAB program for PSF deconvolution. Depending on the data quality, the data may first be smoothed using a median filter with a kernel size of three pixels wide high. The deconvolution program will then be set to perform five iterations of the Lucy-Richardson algorithm using a pre-computed PSF as to allow for convergence.

After deconvolution, the lipid, amide I and amide II maps will be used to determine the expansion of the nuclear envelop with attached endoplasmic reticulum, and the cytoskeleton, respectively. This will produce a visual representation of the morphological changes undergone by the cell. The lipid versus Amide I and/or lipid versus Amide II ratio will then be computed as to arrive at maps with relative concentrations. The results from these ratios will show if the total cellular content of lipid changed compared to the amide I/II content, and provide details on the effects of boric acid on cytoskeleton remodeling and cell growth.

Beamtime required.

30 samples are to be imaged in total, namely the cells grown under 0, 5, 12.5, 25 and 50 mM boric acid at time points 1 through 6. From previous data collections using the Bruker Hyperion 3000 FTIR microscope, approximately 15 hours are required to locate

and individually image 50-70 single cells on each sample. Thus, a total of 450 hours of beam time is required to image all samples from each time point. Therefore, we request 6 days of beamtime per cycle over the next year to complete this study.

Proposed work, plans and accomplishments for Beam Time in the requested cycle for this PASS Form:

(limit 2000 characters including spaces)

During the first beamtime cycle, we will begin by studying the control (0 mM) and the highest concentrations of boric acid treatment. These two concentrations will involve 12 samples, which will require ~7 days of beamtime.

FOR MORE INFORMATION ON THIS RESEARCH:

A.S. Acerbo, L.M. Miller. Assessment of the chemical changes induced in human melanoma cells by boric acid treatment using infrared imaging. *Analyst* **134(8)**: 1669-74.