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3D/4D Multiscale Imaging in Acute Lymphoblastic Leukemia cells- visualizing dynamics of cell death.

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Abstract

Quantitative phase detection is a new methodology that provides quantitative information on cellular morphology to monitor the cell status, drug response and toxicity. In this paper the morphological changes in acute leukemia cells treated with chitosan were detected using d'Bioimager a robust imaging system. Quantitative phase image of the cells was obtained with numerical analysis. Results show that the average area and optical volume of the chitosan treated cells is significantly reduced when compared with the control cells, which reveals the effect of chitosan on the cancer cells. From the results it can be attributed that d'Bioimager can be used as a non-invasive imaging alternative to measure the morphological changes of the living cells in real time.

Keywords: Chitosan, cancer cells, Phase imaging.

Introduction.

Cell morphology, growth and homeostasis are the mirrors of cell function which determine the cell fate in many biological functions. The morphological change is a typical feature to distinguish between apoptosis and necrosis in the process of programmed cell death. Revealing the pathophysiology of disease at the cellular level is essential to understand the mechanism of human disease. In addition to this the influence of living cells by different environmental factors and anticancer drugs can be revealed by the cell morphology. Understanding of cellular events remains incomplete by the limitations of the imaging techniques. As a result several novel optical imaging techniques were developed and their translation into the fields of biology and medicine were witnessed. Among the novel optical imaging techniques Quantitative Phase imaging (1-4) is a highly

sensitive method for studying the cellular morphology and could be employed as a non-invasive and label free method.

Chitosan, a multipurpose biomaterial, has been shown to exert effects against several types of cancer. However, the mechanisms underlying the anticancer activities of chitosan on acute leukemic cells remain largely unknown. The present study was aimed to study the effects of chitosan on the acute lymphoblastic leukemic cells to understand the nature of cancer cell proliferation and to develop personalized therapies; there is a need for new methodologies that provide quantitative information to monitor the effect of drugs on cell growth as well as morphological and phenotypic changes. The results shown here establish that quantitative phase imaging provides biologically relevant information in the process of cell death.

The d'Bioimager imaging system (5) uses Transport of Intensity Equation to extract phase from three or more images in focus. The phase thus retrieved is simulated as a stack to study morphological changes. It is a replacement of the standard camera for any bright field microscope and provides 3D and 4D images of live cells. The d'Bioimager retrieves quantitative phase and hence optical depth based on the Transport of Intensity Equations (TIE). By acquiring the three frames in rapid succession, it provides real-time quantitative phase imaging (3D) over time (4D). The novel optics and algorithm allows for near real-time measurement of phase which can further add clarity to the morphological changes associated with cell death.

Materials and Methods:

All reagents & chemicals were purchased from Sigma -Aldrich company. Chitosan low molecular weight (LMW CS) (85% degree of deacetylation) and sodium tripolyphosphate (TPP) were purchased from Sigma–Aldrich Co (MO, USA). BCL2 (AAA) Jurkat (ATCC[®] CRL-2902[™]) was purchased from American Type Culture Collection (ATCC), USA. The other chemicals utilized to carry out the experiment were of analytical grade.

In vitro measurement of acute lymphoblastic leukemic cells.

The present work was done in acute leukemic cells acquired from ATCC. Leukemic cells were treated with chitosan and maintained at 37°C in 5% CO₂ incubator. After incubating 24 h, cells were gathered for further experiments. Cells were maintained in DMEM medium supplemented with FBS (10%), glutamine (1%), and penicillin–streptomycin (100U/ml) in 5% CO₂ incubator at 37°C.

Quantitative Phase imaging using d'Biomager.

The d' Biomager by d'Optron Pte Ltd is based on the principle of Transport of Intensity Equations. By using a patented optical system, the three images required for use in TIE are captured in quick succession which allows dynamic visualization of live cells. The system can be coupled to any microscope through a C-mount adapter as shown in Fig. 1(a). This microscope uses LED illumination which provides the needed spatial coherence for the system. The accompanying software captures the images in near real-time (Fig. 1(b)) and displays the phase, optical path length and also the DIC images as shown in Fig. 1(c). It also allows saving the raw data for further analysis using other software such as Matlab.

Figure1(a) d'Biomager attached to a standard microscope. (b) software interface showing captured image and ROI selection (c) Processed Phase Image

Results and Discussion

In the present study acute leukemic cells treated with chitosan at two different concentration CS1-30ug/ml ,CS2-60ug/ml and the morphology of the control and treated cells are depicted in Fig. 3 (a,b & c). Control cells are with the shape of polygon, which indicate that cells are in a healthy status. Whereas the treated cells due to cell death gradually becomes rounded under the surface tension in the solution. In the treated cells, not only the surface area changes into a circular shape, but also the cell thickness has a visible change. Therefore, the oversize optical thickness and change in surface area; volume is a characterization of the abnormal cells. With these results observed, we can denote that cells treated with chitosan have obvious changes in the cell morphology.

The size distribution between the control and the treated cells both the cell area and optical thickness implies the shape changes accordingly. The plots of control (C) versus CS1 show an increase in cell radius in CS1 than C and a subsequent decrease in radius in the case CS2. It may be assumed that a flattening effect/ shrinkage may have caused an increase in cell size for CS1. Though not significant, an increase in cellular peak height has also been observed in the case of CS and CS2 when compared to C. Consequently, the volume was significantly lower for CS2 when compared to C and CS1.

The morphological changes observed confirm the significant difference in the area of the cells in the chitosan treated cells than the control cells. This indicates that chitosan at 60ug/ml could cause cellular shrinkage which implies the process of cell death under the influence of chitosan. Further the changes in optical volume and thickness in the chitosan treated cells supports its effect towards anticancer activity and warrants further investigation.

a)

b)

c)

Fig-3a, b & c. –Phase imaging of acute leukemia cells - control and chitosan treated cells (CS1 & CS2) at the magnification of 40x objective.

Conclusion

We have demonstrated a highly sensitive method for drug screening application with d'Bioimager. In addition to the measurement of cellular morphology it can simultaneously provide information on cellular growth kinetics and its motility. Our future study will be to understand and characterize the cellular events that relate the cell death. The biological insight provided by the phase imaging will improve our understanding of the cancer metastasis which will lead to potential drug design and therapeutic effectiveness.

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