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Adaptation of the cytokinesis-block micronucleus assay to imaging flow cytometry

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Abstract

Background:
Following a radiological or nuclear event, a rapid dose assessment can inform medical providers as to the proper course of treatment for exposed individuals. In order to achieve this goal our group has adapted the cytokinesis-block micronucleus (CBMN) to an imaging flow cytometry (IFC)-based method. These two techniques were paired to create an automated high-throughput biodosimetry tool to improve upon the traditional and time consuming scoring microscopy-based method. The CBMN assay is used to assess the frequency of chromosomal damage, specifically micronuclei (MN), in cells where cytokinesis has been blocked without inhibiting nuclear division. These cytokinesis-blocked cells thus produce binucleated cells (BNC) rather than permitting the two daughter cells to separate. Damage inflicted by the radiation can be estimated by quantifying the frequency of MN per BNC derived from human peripheral blood lymphocytes.

Method:
Blood samples from eight healthy donors that are diverse in age (between the ages of 25 to 60 years), sex (3 male and 5 female), and background were irradiated using the X-RAD 320 (Precision X-ray) at an energy of 250 kVp and current of 12.5 mA in the dose range of 0 -10 Gy. Following irradiation, blood samples were incubated for two hours at 37°C, and 5% CO_2 to allow for DNA repair. Then the blood was placed in culture media containing phytohemagglutinin (PHA) which stimulates the lymphocytes to proliferate. After 24 hours of incubation, cytochalasin-B was added to block cytokinesis; this chemical stops the cells from dividing into two daughter cells. Samples were returned to incubation for 44 hours at which time the cells were fixed with FACSLyse which fixes the lymphocytes while lysing the red blood cells. Fixation of 22 samples requires two people and approximately two hours. The cells were then stained with a fluorescent DNA stain (DRAQ5) which is considered to be the most appropiate DNA stain in cell prolifeation studies with the laser wavelength (642 nm) used on our cytometer. IFC analysis of the samples was performed on the IFC ImageStream$^{®X}$ MarkII (ISX-MKII) using an autoplate that can hold up to 96 samples.This plate can be loaded directly onto the ISX-MKII autosampler where analysis can be automatically performed for each dose point. Data files were analyzed using the IDEAS® Analysis Software which determined the total number of events, the total number of binucleated cells (BNC), and the number of micronuclei (MN) per BNC.

Results:
During this methods-development project, several experimental parameters were optimized to improve throughput: 1) the volume of blood drawn from donors was minimized from 1 mL to 0.2 mL; 2) the culture time was reduced from 72 hours to 68 hours; 3) the number of files generated showing the DNA stain on individual cells was minimized; and 4) the analysis template was modified to improve the automatic selection

of BNCs and MN. As a result of improving the methodology, a laboratory specific calibration curve was generated using the ratio of micronuclei (MN) per binucleated cells (BNC). This curve demonstrated a dose responsive increase in MN/BNC frequency up to 5 Gy after which, the MN/BNC frequency started to decrease due to excessive damage in the cells. This curve can then be used to estimate the dose to a sample exposed to an unknown amount of radiation in this dose range.

Conclusion:

The dose-response curve confirms the feasibility of pairing classic CBMN to IFC to provide triage-quality dose estimates in the range of 0 to 5 Gy (± 0.5 Gy), in the event of a radiological or nuclear emergency. By pairing these two techniques, it is possible to eliminate the need for manual microscopy, further reducing the required analysis time from weeks to days. The CBMN assay adapted to IFC can be used as a tool during an emergency event to assess the irradiation dose experienced by individuals to inform the medical community of the appropriate course of treatment. For example, at doses below 2 Gy, the patient would be monitored as an outpatient, whereas at high doses hospitalization and treatment with cytokines and other medical options are required. The next steps for this work includes validating the methodology with blinded samples and performing an interlaboratory comparison with our collaborators, Columbia University. In closing, accurate biodosimetry can be achieved using an automated miniaturized protocol and could significantly improve the throughput of biodosimetry for emergency response.

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