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REVIEW PAPER

Microfluidic Integrated Technology: A Potential Tool for Portable Radiation Biodosimetry

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ABSTRACT

In the event of a mass radiological or nuclear incident, a large number of individuals would require rapid biodosimetric screening for proper medical care, mitigation and follow-up procedures. Mass radiological triage is critical after any such large-scale event because of the need for Dose assessment of suffered individuals at an early stage. With the increasing probability of such unprecedented incidents around the world, the need for modelling and development of new medical countermeasures for potential future chemical, biological, radiological and nuclear has been well established. Unfortunately, the capacity of most of these methods are still restricted to laboratory establishments due to resource limitations, need of high end expertise or general immobility of bulky instruments required for the same. So far there exists no rapid diagnostic technique that may reliably discriminate levels of ionising radiation exposure based on samples collected at a single time point. In classical clinical settings, complete blood count, particularly the lymphocyte count is based on temporal assessment. The diagnostic 'gold standard' in the field of radiation biodosimetry is the dicentric chromosome assay which happens to be highly labour-intensive and extensively time consuming, rendering it inefficient in case of mass casualty situations. Advanced technologies such as microfluidic platform, BioMEMS, μTAS carry the potential to make system highly portable, cost efficient and independent of high skilled expertise. In this review of the latest advances in portable biodosimetry we evaluate our progress and identify areas that still need to be addressed to achieve true field-deployment readiness.

Keywords: Ionising radiation; BioMEMS; Microfluidics; Radiation biodosimetry; Chemilumenescence

1. INTRODUCTION

Radiation biodosimetry is an important aspect of accidental mass radiological triage assessment and combat. While conventional standards for biodosimetry (DCA) prove to be accurate and reliable, however, their resource dependence and labour intensiveness hinders their utilizations as an ideal method for mass casualty assessment and follow-up care. The occurrence of any radiological or nuclear event would lead to an urgent need for qualitative and quantitative dose assessment for follow up medical care and mitigation process management[1,2]. Therefore, it is critically important to identify novel diagnostic biomarkers to ensure effective medical treatment[3] .Variations in the radiation field, heterogeneity of exposure pattern, exposure durations, and uncertainties of total-body *vs.* partial-body irradiation makes it strenuous to establish the level of exposure accurately[4,5]. Mass hysteria or psychosomatically-induced symptoms further complicate the triage procedures during mass casualty events, especially where potential radiation exposure is a major concern. It further suggested the need of independent physiological biomarkers of radiation exposure[6]. Present review explains the advance micro-scaling microfluidics and BioMEMS techniques for faster, smarter and reliable radiation biodosimetric application.

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2. CURRENT METHODS OF BIODOSIMETRY

A radiation biodosimeteric marker can be characterized as a biological molecule with dose dependent expression pattern. This comprises a very large disparate classes of molecules and technology platforms, including DNA, RNA and protein expression, chromosomal aberration (Dicentric Chromosome assay) blood count changes, the metabolites identified by Tyburski and colleagues differed from most of classical biomarkers of radiation exposure related to oxidative damage to DNA structure (e.g., 8-hydroxy-2′-deoxy guanosine thymine glycol and thymidine glycol), which also suggested that gamma radiation may damage DNA differently than other forms of radiation. These potential markers are usually evaluated in blood, plasma, urine or saliva samples that are readily obtainable in a field setting.

3. DISADVANTAGES IN EXISTING RADIATION DOSE ASSESSMENT METHODS

Classical research in radiobiology spans various fields including the identification and quantification of DNA damage, the genes and pathways that are used to repair DNA damage, radiation-induced cell death and signalling pathways used to signal cell cycle arrest, cellular redox homeostasis, lipid and cell membrane damages and the response of mitochondrial

metabolism to radiation exposure[7]. Focus has been shifted towards easily accessible and non-invasive biomarkers identification and validation for radiation dosimetry in order to deviate from the conventional labour intensive techniques. Conventional biodosimetry methods for epidemiologic studies include chromosome aberration, micronucleus assay[8] frequencies from fluorescence in situ hybridisation (FISH) of peripheral blood lymphocytes and electron paramagnetic resonance (EPR) measurements made on tooth enamel. All these techniques require a very high level of precision and experience for accuracy in results and risk management which may not be available in case of a mass casualty.

While, apex medical institutes of the country like AIIMS, New Delhi have introduced workshops on skill development for mass casualty triage for medical care providers in the recent past and simulation devices and software have been adopted[9]. However, concrete steps have not been taken yet to address the CBRN mass casualties. The preparedness of medical care-providers in case of radiation mishaps still paints a hazy picture[10].

The dicentric chromosome assay (DCA) currently the standard tool of choice, but being labour-intensive and time-consuming method[11], it is not the most ideal options for mass casualty management. Automated dicentric chromosome identifier (ADCI) is an image based algorithm software available to assist DCA for quicker analysis. A rapid scoring approach is also being developed which enables working with minimal need for human expertise unable to deliver medical grade diagnosis as the chances of misdiagnosis escalate.

Biomarker assessment by conventional methods is a very elaborate process requires large amount of sample processing time and high level of expertise to achieve higher accuracy in results. Instruments like the cell counter, karyotyping paraphernalia, HPLC machinery, UPLC and Tandem Mass spectroscopy and light microscopes require sophisticated positioning and lighting conditions which may not always be available during mass casualty incident areas of accident.

4. MICROFLUIDICS

Microfluidics is an engineering devices that allow fluid flow to channels smaller than 1 mm in at least one dimension. These devices can reduce reagent consumption, allow well controlled mixing and particle manipulation, integrate and automate multiple assays (known as lab-on-a-chip), and facilitate imaging and tracking. Microfluidics encompasses a vast number of disciplines and is emerging as a potential tool for speeding up processes in the field of biological and chemical assay application. Since microfluidics involves micro scaling of conventional design of devices, there is an expected alteration in physical characteristics of the fluids involved. Keeping these anomalies in consideration, microfluidic devices with a lab on chip model approach are designed.

5. MICROFLUIDIC BASED FIELD BIODOSIMETRY

Microfluidics, that can handle small amounts of fluids in confined and controlled environment, is an emerging field for several years. Some microfluidic devices, though even at early stages of development, may help radiobiological research particularly to study cellular, tissue and total-body response upon irradiation. Microfluidics devices may be used in clinical biodosimetry applications.

6. MICROFLUIDICS AND APPLICATION IN MOLECULAR BIOLOGY

Usage of Arduino based microfluidics chambers for DNA extraction holds immense potential in genomic studies due to decreased run times and increased specificity of overall process due to smoother acceleration and application integration to the systemic process[12]. Genomic studies often require the study at nanomole scaled expression of specific genes. A microarray is usually employed for such studies as it enabled to analyse DNA spots uptofew picomoles. While, microarray is a very helpful tool, it has scope of further improvement with the help of microfluidics. Electrochemical biosensors when engineered with nanomaterials have been proven to showcase higher performance in terms of selectivity, stability and sensitivity. Also, functional nanomaterials when coupled with bio recognition elements can scale up biosensing by many folds[13]. Electrochemical enzymatic and aptamer biosensors or aptasensors, based on the integrated microchip could revolutionise point-of-care diagnostics and environmental monitoring. Furthermore, microfluidics can enhance clinical diagnostic techniques for analyte assays mimicking conventional barcode models and produce high throughput results when coupled with micro sensors. A microfluidics based chamber with multiple microchannels can help to carryout multiple nucleic acid assays in a shorter span of time with higher precision and portability (results may be obtained on a smartphone device). Demonstrative studies have shown great potential with immunoassays for HIV marker proteins[13]. Development of optical array microchip biosensors holds immense potential for label free, expeditious detection of multiple bio-hazardous agents ranging from bacterial spore to proteinaceous toxins. Use of optical microchip sensors enables detection of the changes in refractive index due to antigen-antibody interaction. Whereas, another microfluidic technology includes nanomembrane based nucleic acid sensing platforms hold a promising future for DNA/RNA analysis making it label-free, reagent-free, faster, highly sensitive and the possibility of eliminating the need of PCR all together[14]. Experimental results have shown the major cost efficiency for upscalling potential of microfluidics when coupled with optics and electronics[15]. Microfluidic tectonics is a comprehensive construction platform for microfluidic systems that can help to develop an easy to read colour change and disposable ELISA for detection of toxins like botulinum utilisation of optical detection provides a cost efficient module for lab-on-chip devices owing to be robust, precise and cost efficient devices[16].

Though, gel electrophoresis has been established as an excellent technique in molecular biology. However, a newer and faster technique has also been developed called capillary electrophoresis to scale down the process in dimensions and time of performance simultaneously. Microchip electrophoresis

is a miniaturised platform of capillary electrophoresis with high-throughput, rapid analysis time, lowered consumption of sample and solvent, making it highly cost effective[17]. RT-PCR is one of the most precise analytical tools and a golden standard for the identification of diseases like H1N1. However, it is time and labour intensive upto some extent. Development of wire guided droplet manipulation and computational fluid dynamics has enabled the completion of a PCR reaction of thirty cycles (ideal run time of 2 h) in less than ten minutes[18]. These techniques when employed in biodosimetry could revolutionise the field of radiation dosimetry, with minimising the processing time along with reducing the dependency on trained professionals.

7. CHEMILUMINESCENCE AND MICROFLUIDICS

Application of luciferase immunoprecipitation systems (LIPS) in microfluidic format for point of care diagnostics of infectious and autoimmune diseases has shown impressive results with up to 100 per cent sensitivity and specificity in experimental researches with HSV-2 infection diagnosis[19]. The use of transparent microfluidics based reaction chip has been designed to enable lense-less portable imaging for diagnostic purposes using multiplex bioanalysis[20]. This could help in early detection of infectious agents as observed for parvovirus B19 DNA. This device can be of aid for early detection and follow up care of diseases as well as help in estimation of micro dosages in dosimetric analysis leading to a better treatment (Fig. 1). Similar type of approach can be used to to detect and validate radiation biomarker gene or protein expression using microfluidic device with precision time and sensitivity.

The portable MOA instrument consist of four wafer sandwich which integrates high voltage CE power supplies, pneumatic controls and fluorescence detection optics necessary for field operation as shown in Fig. 2. The amino acid concentration sensitivities range from micromolar to 0.1 nM, corresponding to part-per-trillion sensitivity.

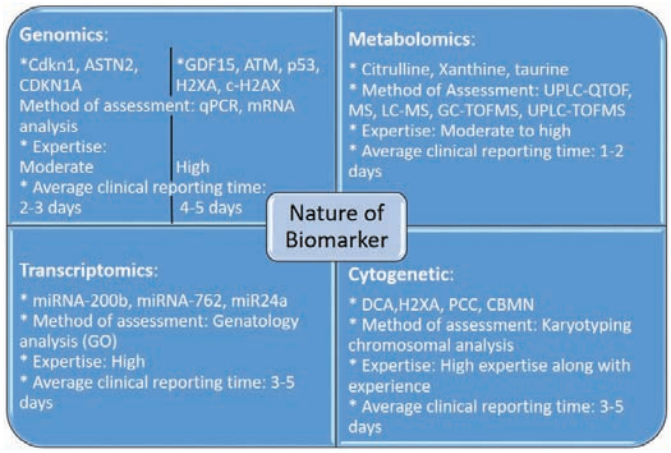
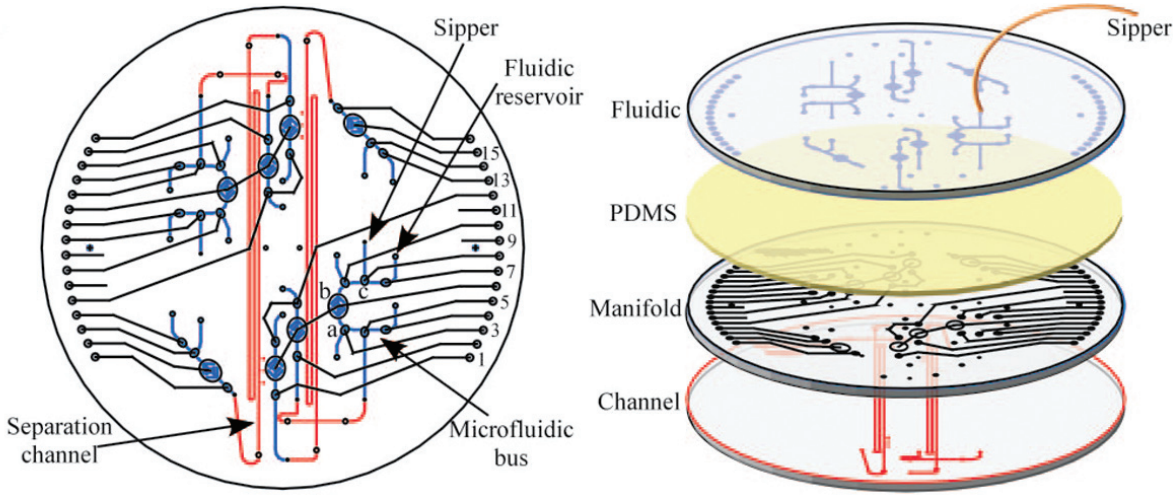


Figure 1. Different classes of Biomarkers involved in current radiation biodosimetry.

8. BIODOSIMETRY BASED ON GENOMICS AND TRANSCRIPTOMICS

Phosphorylated histone γ-H2AX foci has been an established radiation biomarker that form at the sites of DNA double-strand breaks..γ-H2AX phosphorylation was found to be highly linear with radiation doses and thus currently in use for radiation biodosimetry at laboratory level. To transform the conventional γ-H2AX foci scoring techniques into high-through-put microfluidic based technique, researchers at Oxford University have developed a portable microfluidic fluorescence spectrometer. This device can records and analysed 10,000 spectra of γ-H2AX phosphorylating foci in just approximately 6 min. Another group developed a microfludic device which is capable of evaluating the radiation damage of cells by measuring the ratio of the number of cells with γ -H2AX fluorescence signals to the total numbers of cells in the sample. The simple prototype of this developed microfluidic flow cytometer can detect fluorescent signals corresponding to a radiation dose rate as low as 0.95 J/m^2, which is very close to the detection limit of a commercial confocal microscope, 0.75



(a) (b)

Figure 2. The Mars Organic Analyser (MOA), a microfabricated capillary electrophoresis (CE) instrument for sensitive amino acid biomarker analysis.

J/m^2. The developed device has many advantages such as low cost, easy operation and portability[21].

Similarly, a group of researchers at the Center for High-Throughput Minimally Invasive Radiation Biodosimetry at Columbia University Medical Center, New York, have developed a fully automated high-throughput system called RABIT (Rapid Automated Biodosimetry Tool). This system allow to measure γ-H2AX yields from finger stick-derived blood samples. These mobile workstation uses purpose-built robotics, lymphocyte handling to fully automatic γ-H2AX immune-cytochemical protocol. Robotic biodosimetry workstation was able to detect dose-dependent increase in γ-H2AX fluorescence in irradiated (0-8Gy) peripheral blood samples.

It was reported earlier that a small group of genes, i.e. ASTN2, CDKN1A, GDF15 and ATM, were found to be highly predictive of radiation exposure. Expression levels of these genes were highly predictive up to 6 Gy dose of radiation[22,23]. Another study evaluated cytokine levels in saliva from patients undergoing head and neck radiotherapy. A dose-response relationship was observed for expression of VEGF, MCP-1,EGF, IL-4, IL-6, IL-8, and TNF-a in radiotherapy patients[22,23]. Similarly, miRNA signatures serve as a proof-of-concept for their utilisation as biomarker of radiation injuries[24]. Microfluidic technique can speed up detection and validation of all these radiation biomarker candidates.

9. CONCLUSIONS

It is evident from the above assessments that while conventional radiation biodosimetry techniques are reliable to a great extent, however, an urgent need for faster, smarter and reliable point of care diagnostics is prominent. Conventional methods have their limitation like time, resource and labour intensive. In a densely populated country like India, the preparedness towards CBRN accidents is an unavoidable necessity. Although, DCA, blood based analysis and EPR continue to reign as recognised medical standards for radiation dose assessment. The use of microfluidics and BioMEMS emerges as an excellent tool to aid conventional dosimetry techniques and speeding up channels to fine tune the metabolomics and genetics based biodosimeter analysis. Microfluidics is helpful in making complex, time dependent analytical techniques and thus promising option to expedite radiation biodosimetry for emergency applications.

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He has contributed throughout in designing the frame of article and guidance and has reviewed the manuscript.