Published in final edited form as:

Cancer Lett. 2016 February 28; 371(2): 292–300. doi:10.1016/j.canlet.2015.11.033.

Microfluidics as a new tool in radiation biology

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Abstract

Ionizing radiations interact with molecules at the cellular and molecular levels leading to several biochemical modifications that may be responsible for biological effects on tissue or whole organisms. The study of these changes is difficult because of the complexity of the biological response(s) to radiations and the lack of reliable models able to mimic the whole molecular phenomenon and different communications between the various cell networks, from the cell activation to the macroscopic effect at the tissue or organismal level. Microfluidics, the science and technology of systems that can handle small amounts of fluids in confined and controlled environment, has been an emerging field for several years. Some microfluidic devices, even at early stages of development, may already help radiobiological research by proposing new approaches to study cellular, tissue and total-body behavior upon irradiation. These devices may also be used in clinical biodosimetry since microfluidic technology is frequently developed for integrating complex bioassay chemistries into automated user-friendly, reproducible and sensitive analyses. In this review, we discuss the use, numerous advantages, and possible future of microfluidic technology in the field of radiobiology. We will also examine the disadvantages and required improvements for microfluidics to be fully practical in radiation research and to become an enabling tool for radiobiologists and radiation oncologists.

Keywords

Microfluidics; Radiation research; Radiobiological models; Organ-on-a-chip; Biodosimetry markers; Cancer

Difficulties encountered by radiobiology models

Radiobiology is the area of medical science that studies the action of ionizing radiation on cells, biological tissue, and living organisms. Several years ago, the paradigm of

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

radiobiology was that the biological effects of ionizing radiation occurred only in the nucleus of cells crossed by particles, and that cell death or dysfunction was strictly due to unrepaired or misrepaired DNA [1]. Now, this DNA-centric view is obsolete and numerous results have shown the importance of non-DNA centered effects, macro- and microenvironments, hierarchical organization, underlying genetics, evolution, adaptation and signaling at all levels, from cell to organism (Fig. 1) [2]. Therefore, classical research in radiobiology focuses on various fields including the identification and quantification of types of DNA damage, the genes and pathways that are used to repair DNA damage, radiation-induced cell death, and also the signaling pathways used to signal cell cycle arrest, cellular redox homeostasis, lipid and cell membrane damages, and the response of mitochondrial metabolism to radiation [3].

Hence, the cellular response to radiation is complex and not only centered on the nucleus, with many interactions occurring between new molecules generated by irradiation and the whole of cellular components. To study these mechanisms, it is necessary to have models able to combine a good biological representation with good handling and receptivity to irradiation. This is especially true when biological effects of radiation need to be investigated on a macroscopic scale, both on the tissue and organismal levels, such as the development of small molecules that target tumor hypoxia, tumor radioresistance, or the mechanisms of dose-limiting normal tissue effects. For example, most current cellular models have limitations reporting the real cellular response to radiation and mechanisms involved in tumor radioresistance. In addition to the multiple events at the cellular level described above, these models must also take into account the *in vivo* tumor heterogeneity and its interaction with the microenvironment [4]. In the same way, the study of radiation-induced toxicity of normal tissue is also complex due to the response mediated by various cell types, including inflammatory, stromal, endothelial, and parenchymal cells, actively responding through the release or activation of downstream cytokines, growth factors or chemokines [5]. The bystander effect, which is a phenomenon that refers to the radiation-induced effects in neighboring cells by indirect exposure from the ionizing radiation field, is one example that illustrates the complex biological response to irradiation. Although this phenomenon has been observed for several years, the actual cellular mechanisms and their consequences at the organismal level are not yet understood [6].

Microfluidics may help radiobiology ... and vice-versa

Microfluidics, the field of science and technology that deals with systems that process and manipulate small amounts of fluids, can provide reproducible and controllable environments mimicking *in vivo* systems [7]. Over the years, this technology has generated a lot of expectations among the scientific community, particularly as an approach to develop platforms that enhance the capabilities of investigators in biological and medical research [8]. Unfortunately, despite material advances in microfluidics as a technological platform, the adoption of novel microfluidic techniques in mainstream biology research has not matched the initial enthusiasm surrounding the field, leaving some people arguing that the technology is still in search of a “killer application” which greatly outperforms current methods [9].

However, the specific features of models used in radiobiology, which need to control both biological and molecular aspects, and the physical approach of the irradiation, might allow microfluidic technology to find a new “niche application”.

As it was reported above, radiobiology must have complex models able to represent the different types of cells and their interactions and able to easily handle different categories and conditions of irradiation. Thus, *in vivo* models are the best way to represent the biological reality compared to *in vitro* cellular models, but they usually require expansive and sophisticated equipment for irradiation. Conversely, *in vitro* models can be more easily irradiated but are limited in their ability to reproduce the complex cellular response to radiation. Microfluidics, with the capacity to maintain viable and functional tissue biopsies or to mimic the natural physiological response of an organ with the emerging organ-on-a-chip technology, may be a good compromise between the ease of irradiation and the functional capability to study real biological responses.

Besides the development of models for fundamental and biophysical research, there is another field of investigation for microfluidics in translational radiobiology research: biomarker assay development. Biomarkers are becoming a cornerstone in precision medicine, and there is no exception in radiation research. Especially in radiation therapy, there is an urgent need to identify biomarkers that are able to predict tumor radioresistance or radiation-induced toxicity of normal tissue, evaluate response to radiation treatment protocols and monitor its clinical success, or determine absorbed ionizing radiation dose after a radiologic or nuclear event, using a sensitive dosimetric biomarker. These markers may allow the prescription of optimal, individually-tailored treatment plans. However, despite the numerous studies and discoveries in this field, no robust biomarkers have been validated for the clinical setting. As such, microfluidics may offer two important opportunities: first, it can address some of the biomarker development obstacles, thanks to its capacity for high-throughput screening, its high efficiency and its potential to achieve higher sensitivities. Second, it can provide the precise and complex platform required to bridge the gap between biomarker research and the biomarker-based analytical device market, thanks to the low sample and reagent requirements, the fast analytical times and its portability for point-of-care applications [10].

Although few applications are used in radiobiology, we will describe in this report what kinds of devices have already been tested and might have a promising future for validation until becoming inescapable in this domain (Table 1). We will discuss their advantages compared to traditional techniques and possible inconveniences which may slow their translational validation.

***In vitro* applications**

Despite of few attempts developing three-dimensional *in vitro* models, such as 3-D tumor spheroids [17], radiobiology still uses standard cell culture techniques for investigating the molecular and cellular mechanisms involved in radiation response. Computational models are also needed for gathering observations from these standard cell culture systems for resolving the differences between *in vivo* tissue response and *in vitro* data [18]. However, all

properties of microfluidics allow developing *in vitro* models mimicking ever closer the parameters of *in vivo* conditions. One of the most favorable benefit of using microfluidics for biology research is the ability to control the local cellular microenvironment precisely, without (or with less) interference from the external environment. A controlled cellular microenvironment enables better monitoring of cellular behavior [19]. Therefore, microfluidic devices are becoming versatile *in vitro* platforms to provide highly controlled environments and that can be configured in different cell-culture formats [e.g., two-dimensional (2D) or three-dimensional (3D) cell culture, co-culture, single-cell culture and automatic cell culture] for studying various biological systems and evaluating the cellular response to drugs or other stressors. For example, microfluidic devices are currently used as co-culture system for studying the interactions between tumor cells [20–23], or bacteria system [24], or neighboring normal cells for characterizing the effects of new drugs [25,26], but also for clinical diagnostic applications [27] or in assisted reproductive technology (e.g., *in vitro* fertilization) [28]. The use of microfluidics for *in vitro* cell culture allows single cell level quantification of cellular responses to internal or external stimuli with a high temporal resolution over prolonged cell culture periods. The concentration of biomolecules can be controlled spatially and temporally, thus allowing for instance the generation of diffusion-based gradients of given molecules [29]. As such, microfluidics is widely used for studying resistance to chemotherapy, screening and optimizing drug combinations, and monitoring cellular responses [30–34]. However, few studies have used microfluidics to evaluate the effects of ionizing radiations on cells.

Initially, Carr et al. used microfluidic cartridges to detect radiation-induced cell death of human head and neck squamous cell carcinoma (HNSCC) biopsy samples, as measured by levels of lactate dehydrogenase (LDH), cytochrome c, and caspase-3 cleaved cytokeratin-18 [11]. Rat liver samples were first used to optimize the methodology before using the device for HNSCC or radiation studies. Results showed that the device was able to maintain both the rat liver and HNSCC samples without radiation treatment in a viable state for 15 days, as indicated by minimally detectable LDH levels. To study radiation-induced cell death in human tumors, HNSCC biopsies were then implanted in the device, incubated for 24 hours, and irradiated with single doses between 2 Gy and 40 Gy or a fractioned course of 5×2 Gy over five days. Soluble markers of apoptosis, LDH and cytochrome c, were measured in the microfluidic effluent, but were not effective in detecting radiation-induced cytotoxicity in the tissues. However, a significant increase of apoptotic index (AI), calculated using the immunostaining of caspase-3-cleaved cytokeratin 18, was observed 72 hours after irradiation compared to the untreated samples. Interestingly, results also showed no significant difference in the AI for non-irradiated tissue maintained in the device compared to non-irradiated tissue maintained in *in vitro* cell culture conditions. Unfortunately, this study did not compare the tissue behavior in the microfluidic cartridge in relation to the same tissue in classic cell culture after radiation treatment, and additional studies are needed to investigate this point. By architecting a pseudo *in vivo* three-dimensional environment in a microfluidic device, the authors managed to maintain biopsies of HNSCC in a viable state and to demonstrate the relationship between radiotherapy dose and radiation-induced cell death using tissue-based markers of apoptosis. The use of this device and the detection of caspase-3-cleaved cytokeratin 18 may have a promising future to predict radioresistance of

HNSCC tumors prior to commencement of treatment, even if further study must be done to correlate these results with outcomes in patients before the technology could be validated for its clinical use.

This study demonstrated the capacity of a microfluidic device to maintain viable tissue as good conditions as in the classical *in vitro* techniques, but it is still unclear whether cells in the device react in the same manner as their *in vivo* counterparts. Using organ-on-a-chip technology, a recent study has managed to obtain similar results after radiation treatment using an *in vitro* microfluidic device as found using *in vivo* tissue models.

Indeed, Torisawa et al. engineered bone marrow (eBM) that retains normal *in vivo* cell proportions *in vitro* for at least one week and models both marrow radiation toxicity responses and protective effects of radiation countermeasure drugs, unlike conventional bone marrow culture methods [12].

The bone-marrow-on-a-chip was made by filling a poly(dimethylsiloxane) cartridge's central cylindrical cavity with a type I collagen gel containing bone-inducing demineralized bone powder and bone morphogenetic proteins and sealing the top to restrict access to the hypodermis and reduce adipocyte content in the marrow, before subcutaneous implantation in mice. After growth of the marrow for 8 weeks, *in vivo*, the eBM was then transplanted to an *in vitro* microfluidic device for the easy delivery of nutrients, chemicals, and soluble markers.

The *in vivo* engineering of the bone marrow produced an almost identical sample to natural bone marrow in mice. The in-cartridge marrow was indistinguishable histologically from natural bone-marrow of mice: the ordered internal trabecular network and calcium and phosphorous content of the eBM were very similar to natural mouse bone. In eBM harvested at 8 weeks after implantation, key hematopoietic regulators were localized in normal positions and the distribution of hematopoietic stem cells (HSCs), hematopoietic progenitors, and differentiated blood cells was nearly identical to natural bone marrow. These results all point to the success in forming an engineered bone marrow niche through this method of subcutaneous implantation of a microfluidic device in mice.

There was no difference in cell viability after four or seven days of eBM incubation in the microfluidic device compared to traditional stroma-supported culture with incubation periods for the same amount of time. However, the distribution of HSCs and hematopoietic progenitors was very similar to freshly harvested bone marrow for the eBM cultured on-chip for up to seven days, whereas the marrow on stroma-supported culture showed significant decreases in long-term HSCs and increases in hematopoietic progenitor cells. Remarkably, the cells maintained under microfluidic flow *in vitro* remained great models for natural marrow for at least one week, even without the addition of exogenous cytokines, which are needed for traditional marrow culture. To test self-renewal and differentiation of cultured on-a-chip cells, eBM from the device after four days of culture, and freshly harvested natural mice femur bone marrow were transplanted into lethally irradiated mice. Engraftment and repopulation of all differentiated blood cell lineages occurred at similar rates for both samples, measured at 6 and 16 weeks after transplantation, showing that the

eBM remained functional and multipotent after four days of culture in the microfluidic device. Results were not reported for eBM cultured for seven days, and this should be studied to confirm the functionality and multipotency of these cells for a longer period of time.

The bone marrow-on-a-chip was also shown to react the same physiologically as natural living bone marrow in response to radiation toxicity and a radiation countermeasure drug, whereas marrow cells maintained in stroma-supported cell culture did not. Live mice, the bone-marrow-on-a-chip, and stroma-supported cultured marrow were irradiated with 1- and 4-Gy doses of γ -radiation. Four days later, the proportions of HSCs, hematopoietic progenitors, lymphoid cells, and myeloid cells of the chip marrow were extremely similar to the natural mice marrow, whereas cells cultured on stroma were all much more resistant to the effects of radiation toxicity. To test the response of in-cartridge marrow to a radiation countermeasure drug, granulocyte colony-stimulating factor (G-CSF), which has been validated to prevent post-radiation bone marrow failure, was added to the marrow-on-a-chip one day after radiation exposure. The cells were analyzed three days after treatment with G-CSF. The results matched what had been previously reported *in vivo*, and there was a significant increase in the total number of hematopoietic stem cells and hematopoietic progenitor cells in the treated samples compared to the untreated marrow chips. Unfortunately, these results were not compared to traditional stroma-supported cultures.

Creating bone marrow-on-a-chip is time and labor intensive as the device must be implanted and the marrow grown *in vivo* for eight weeks before being cultured in the microfluidic device. This requires skilled technicians and the ability to perform any experiments on the mature marrow within one week of harvesting the eBM. Despite these inconveniences, this method offers advantage of mimicking human response in the study of radiation *in vitro* compared to traditional cultured marrow cells. The eBM responds, as the organ typically would, *in vivo*, supporting HSCs and progenitor cells in normal proportions. Therefore, the bone marrow-on-a-chip could replace whole animal models with an *in vitro* approach. The microfluidic approach allows for analysis under flow, helping regulate marrow physiology and permitting the study of pharmacokinetic and pharmacodynamics drug behavior. The eBM's hematopoietic niche behaves autonomously and provides a cost-saving benefit, as expensive exogenous cytokines are not needed for the chip like they are in traditional culture. Also, the individual cell populations within the chip can be manipulated to better suit individual experiments through genetic modification, drug manipulation, or insertion of other cell types such as tumors. In the future, similar techniques may be used to grow human marrow in a cartridge, by using immunocompromised mice with human hematopoietic cells, allowing for a near perfect model for precisely monitoring the effects of radiation on humans. Perhaps, other organs may be developed on-chip as well, increasing our ability to study the effects of radiation on many parts of the body *in vitro* [7].

A number of laboratories have also developed techniques for microbeam irradiation of individual cells. These approaches are designed to remove much of the randomness of conventional methods and allow the nature of the targets and pathways involved in a range of radiation effects to be studied with greater selectivity. Another advantage is that the responses of individual cells can be followed in a time-lapse fashion and, for example,

processes such as “bystander” effects can be studied clearly [35]. Because microbeams use narrow beams of radiation, with micrometer or submicrometer dimensions, microfluidic devices can be valuable tools for these studies at these suitable length scales.

Thus, in 2011, Garty et al. used microfluidics to manufacture a novel microbeam using Flow-And-ShooT technology (FAST) [13]. The microfluidic cartridge is used in combination with the permanent magnet microbeam (PMM) in order that cells, tracked by a high-speed digital camera, can flow through a 200 μm wide and 20 μm thick microfluidic channel and intersect with the microbeam radiation, allowing for a high throughput manipulation of irradiated cells. The authors expect the system to reach a throughput of up to 100,000 cells per hour compared to the current microbeam systems which have limited throughputs of about 10,000 cells per hour, preventing the possibility to probe rare endpoints such as mutagenesis and oncogenesis. However, currently, the experiments have only simulated irradiation of about 3000 cells per hour. Faster imaging using a more sensitive cooled CMOS camera is expected to increase this rate, but further work must be done to fully achieve the expected high-throughput results. Beam scattering is another potential problem with this system. However, nominal channel thickness of 20 μm is used to minimize this issue.

The addition of microfluidics into the microbeam end station offers additional flexibility for integrating the beam with other technologies: cell sorting and cell encapsulation. Currently, in microbeam systems, cell sorting is performed by manual cell manipulation with pipettes (at a speed of about 60 cells per hour). This process is very laborious and impractical, when dealing with larger numbers of cells. The integration of microfluidics into the microbeam end station allows for the easy integration of microfluidic cell sorters. Moreover, it has recently been shown that cells can be encapsulated in aqueous droplets submerged in microfluidic channels filled with oil, isolating the cells from the surrounding environment [36]. The encapsulation of cells in droplets can easily be integrated before the cells flow into the FAST microbeam, and the cells can be irradiated in the droplets as they flow past the microbeam. Thus, factors secreted by the cell during irradiation can be captured in a small volume and studied. For example, two or more cells can be encapsulated within the same droplet, one of which can be irradiated and kept in close proximity to the other cells. This would allow bystander signal enhancement due to the close proximity of the cells and the inability of secreted factors to diffuse away. Another advantage of the FAST system is the elimination of the problem of adherence, as cells need not adhering to a membrane as in previous microbeam systems, but flow through a cartridge, allowing for the irradiation of cells in suspension such as lymphocytes and other hematopoietic cells. The irradiation of these types of cells may allow for many new types of experiments, and if the system attains its expected throughput, it will permit studies to gain much higher statistical power, improving the significance of studies in the field of radiobiology.

***In vivo* applications**

Only very small animals and organisms can be studied using microfluidic devices. Most studies reported in the literature use *Caenorhabditis elegans*, although some of them also used drosophila embryo [37]. *C. elegans* is a small semi-transparent nematode, optically

accessible, which is becoming a powerful model for studying a wide variety of biological phenomena and disease models [38]. Many of the properties that make *C. elegans* a useful model organism also make it amenable to manipulation in confined microfluidic channels [39]. A number of novel microfluidic devices using *C. elegans* have been published for studying its behavior under different stimulations [40–42]. Immobilization without anesthesia is one of the main advantages for using microfluidic devices in studying *C. elegans*. Indeed, anesthesia may have several detrimental or uncharacterized side-effects that can interfere with many biological processes in physiologically active animals. Due to the spatial confinement of the microfluidic channels, it is easier to immobilize the animals without the use of anesthesia and minimizing any physiological effect.

The first study on the effects of ionizing radiations in *C. elegans* was performed by Herman et al. in 1976 [43], who analyzed chromosome rearrangements induced by X-rays to produce a balancing lethal system using chromosome balancers such as *Drosophila* [44]. Since then, *C. elegans* became an *in vivo* model system widely used in the field of radiation biology for studying radiation response in germ cells, investigating cellular behavior, oxidative stress or bystander effect [44–46].

The combination of microfluidics and *in vivo* radiation models is unusual. However, microfluidic devices may be useful for microbeam research in order to perform high-throughput studies on the biological effects of targeted ionizing radiation *in vivo*. Thus, Buonanno et al. have implemented a microfluidic tool for microbeam irradiation of *C. elegans* in order to study the radiation effects on DNA by assessing Rad51 foci [14]. The authors developed a microfluidic cartridge with 16 tapered channels to mechanically immobilize 16 *C. elegans* for high-throughput microbeam irradiation without the use of anesthetics, glue, or cooling which likely interfere with normal physiological processes. The microfluidic cartridge served as a “worm clamp”, using liquid flow to transport the nematodes to the microchannels which decreased in size to 10 μm so young adult worms, which are typically 50 μm in diameter, are physically immobilized. Once immobilization occurred, the worms were successfully exposed to a 4.5-MeV proton source, with a beam diameter of 5 μm . DNA damages have been studied by assessing Rad51 foci formation upon the release of the worms from the device by reversing the flow direction. Rad51 foci formation was analyzed by immunohistochemistry 1 h after radiation exposure. Compared to controls, microbeam-irradiated worms showed an increase in Rad51 foci formation. To further confirm that foci formation was induced by proton irradiation, worms were then exposed to 0 or 10 Gy from broad-beam protons. Compared to respective controls, broad-beam-irradiated worms also exhibited an increase in Rad51 foci formation that was similar to that observed in microbeam-irradiated samples. This study shows a novel application of microfluidics with living systems in order to more efficiently study radiobiology. In this case, microfluidics made the microbeam irradiation process much more rapid, allowing the irradiation of many living organisms in parallel, while maintaining precision.

Toward the automation and integration of assay chemistries for biodosimetry markers

In radiobiology, assays for dosimetry biomarkers are important, especially for assessing the radiation dose received and managing triage during a radiological incident [47]. Cytogenetic assays are the most extensively used biodosimetry assays, such as premature chromosome condensation (PCC) assay, micronuclei formation scoring, translocation and dicentric assay together [48]. Dicentric assay is currently the gold standard dosimetric method. It is based on the scoring of dicentric chromosomes (a chromosome with 2 centromeres) which can appear after a misrepair of 2 chromosomes and the abnormal chromosome replication during repair of DNA strand breaks after irradiation. Although radiation induces many other types of chromosomal changes, dicentrics are considered the most sensitive and most specific for assessing radiation dose [49]. However, the dicentric assay is time-consuming and data analyses are challenging while its accuracy is also limited in the domain of doses in excess of 5–8 Gy due to impaired cell proliferation [50]. With the advent of new technologies, a better comprehension of cellular mechanisms involved in the biological response to radiation has been achieved. As a result, several researches have been able to identify molecular markers that may reflect absorbed radiation dose, correlating it with inhibition, reduction and/or over-expression of an ever-expanding number of genes [51–56], miRNA [57,58] or proteins [59] and that might overcome some of the limitations of the commonly used dicentric assay.

Furthermore, microfluidics is suitable for automating molecular biomarkers analysis [10] as an emerging platform for cancer diagnosis [60]. Now, routine lab methods such as PCR [61–63] or ELISA assay [64,65] can be integrated in microfluidic systems. As such, microfluidic technology might help support this field by producing portable devices, rapid, accurate, that could be deployed at the point-of-contact enabling more efficient management of critical medical countermeasures.

In 2010, Brengues et al. developed a biodosimetry quantitative nuclease protection assay (qNPA) and showed how qNPA can be adapted to a microfluidic format in order to be used for rapid high-throughput triage in the case of a nuclear accident [15]. First, the benchtop qNPA was optimized using a previously characterized inflammation assay. Thus, the protocol time was reduced from 27 hours to 9 hours. The benchtop qNPA was then integrated in a microfluidic platform so that the assay could be portable and automated. The microfluidic device was designed using two cartridges – a frontend cartridge for sample preparation and a back-end cartridge for microarray chemiluminescence detection. The same inflammation assay used during optimization was performed using the frontend cartridge before transferring to a microarray plate for detection. This yielded similar gene expression profiles as assays ran completely on the 96-well plate benchtop system, indicating the success of using an integrated microfluidic platform. The authors then tried to adapt qNPA as a biodosimetric assay. They selected 14 genes from an initial discovery set of 74 response genes to be used in a “radiation array” based on previous studies identifying biodosimetric gene signatures using whole genome microarrays. Then, they determined the capacity of qNPA, using a direct lysis of peripheral blood samples without the need for RNA

amplification or labeling, for detecting the biodosimetric signatures. Aliquots of blood from healthy subjects were irradiated with 0, 2, and 8 Gy doses of radiation and cultured for 24 hours. mRNA was then purified from the human whole blood samples and benchtop qNPA assay was performed. A similar gene expression profile was seen in different subjects, and it was possible to distinguish between irradiated and non-irradiated samples based on the results of the assay.

However, further integration of the workflow process is still required until a microfluidic cartridge could fully automate this qNPA process. A major limitation in microfluidics is centrifugation to remove debris from the lysate, so a method to replace the centrifugation step for dealing with whole blood on the cartridge must be developed. Although CD-like microfluidic cartridges have been reported in the literature, they often require more stringent instrumental interface while not necessarily offering comparable centrifugal speed and performance than the ones achievable by benchtop systems [66]. This difficulty may be overcome by using an alternative sample preparation using a device to separate blood cells before starting the qNPA assay. Brengues et al. published results of a microfluidic device that did separate white blood cells from whole blood to be used in gene expression assays [67]. qNPA assay was then successfully run in combination with this approach for the cell separation, but it still needs to be tested with the radiation array and the original front-end cartridge. Also, after the automated cartridge would be fully developed to complete all steps of the assay from whole blood to gene expression signal detection, it will still need to be validated with many more human subjects.

Despite these difficulties in engineering complex features for the device to automate the specimen workflow process and molecular assay detection, the use of microfluidics in biodosimetry would be advantageous for many reasons. It would allow for a point-of-care assay that could be done “in the field” in the event of a nuclear incident. It would allow an untrained person to run the test unlike standard laboratory procedures that require a skilled technician. The use of microfluidics also decreases the amount of patient sample needed, allowing for the test to be accomplished with only a small volume of biological fluid, for example blood collected with a fingerstick. The microfluidic cartridge is expected to be very quick (take less than 12 hours), cheap (cost less than \$7 per sample) and have a high throughput (200,000 samples per week) since it could be deployed at many locations (e.g. hospitals, police stations). As automation of the sample preparation by microfluidic technology can be broadly configured to a multitude of biomarkers panels and assay kits, it is foreseen that commercial systems will leverage diverse applications from pathogens detection to rapid DNA human identification and other medical diagnostics [68]. The technology still needs major improvement and validation, but it seems advantageous to continue to improve microfluidics for biodosimetry purposes.

In a different attempt to create a microfluidic biodosimeter, Pope et al. designed a miniature fluorescence cytometer prototype using microfluidics to measure the intensity of the fluorescent signal from immunofluorescent labeled γ -H2AX [16]. The fluorescence intensity of this histone is directly related to the radiation dose, providing a method of rapid evaluation of radiation dose. Currently, flow cytometry is the most commonly used method of radiation biodosimetry. However, this method is complex, requires regular maintenance

and calibration, and needs a skilled technician for operation and interpretation of the results. In contrast, a microfluidic fluorescence cytometer would be easily automated and does not require a skilled operator, making it highly useful for catastrophic situations. Thus to begin development, simulations were performed and un-mixing algorithms were developed to create a dose–response curve of the immunofluorescent labeled γ -H2AX. Then, a prototype microfluidic device was created and tested. Human lymphoblastoid cells in culture were irradiated with 0–8 Gy doses and incubated for 1 hour before being fixed, permeabilized, and stained for γ -H2AX detection. The samples were then excited with light at 488 nm, and fluorescence emission was recorded at 495 nm. The tests performed on cultured lymphoblastoid cells showed promising results and resembled the simulation studies, but further work must be done to validate the use of a γ -H2AX assay for rapid biodosimetry. Studies must be done to determine dose–response at different post-exposure times, determine if results differ when irradiation occurs *in vivo*, and identify possible variation between individuals. The cost of the laser, spectrometer, and syringe pump is high, but these items could be reused. Another potential problem is that γ -H2AX foci are lost quickly overtime, effectively limiting the use of this assay to a period of a few days following radiation exposure. However, despite these limitations, the ability to quickly analyze radiation exposure through the use of a portable microfluidic cartridge would greatly assist in initial triage after a large radiological incident and therefore improve overall outcomes. The prototype acquired 10,000 spectra in 6 minutes, offering a very quick analysis speed in the event of an emergency. In 2014, Wang et al. fabricated a similar microfluidic device with the same purpose as Pope [69]. This system is extremely efficient as only 20–50 μ L of blood is needed to obtain an accurate radiation dose measurement compared to 2–5 mL in current commercial flow cytometers. However, Wang’s device was tested with UV radiation at 240 nm, not ionizing radiation, but this study proves again the utility of microfluidic device for assaying γ -H2AX as a biomarker for radiation biodosimetry. This suggests that both microfluidic fluorescence cytometers could be used for a range of biodosimetry purposes including triage in the case of large scale human exposures to ionizing radiation and personalized risk analysis of radiation treatments.

Conclusion

Radiobiology is just starting to realize the full potential of microfluidics to advance its study of radiation *in vitro*, *in vivo*, and for biological dosimetry (Fig. 2).

Currently, studies on chemotherapy effects using microfluidic devices are more developed than radiotherapy studies, certainly due to difficulty in radiation management and accessing an irradiator. Nevertheless, the organ-on-a-chip technology seems to be the most promising *in vitro* approach using microfluidics in the radiobiology field, and its use will increase in the coming years. Results from previous studies prove the efficiency of microfluidic devices compared to the classical *in vitro* cell culture and *in vivo* experiments. This may allow this approach to be used in fundamental biomedical research or for pre-clinical studies to obtain preliminary results more robust than with classical *in vitro* methods while avoiding complex *in vivo* models which need important infrastructures and a small animal-adapted irradiator. However, it should be noted that equipment or development of this technology may require expensive cumbersome instrumental interface.

The validation step on small animals, especially in pre-clinical studies, still seems to be unavoidable and microfluidic use is difficult because it is not adapted to a small animal scale. Therefore, *in vivo* applications seem to be currently only reserved for microbeam irradiation. However, combined with the advantages of microbeam studies (highly-focused radiation beam leading to great selectivity, precision and accuracy), microfluidics can bring many other benefits for *in vivo* microbeam applications like multiplexing, minimal sample manipulation or reduced use of drugs that might interfere with normal physiological processes.

One of the most important advantages of microfluidic systems is their capability to process extremely low volumes of sample and reagents, which significantly reduce the cost of assays [70]. This is particularly important in the case of irradiated patient samples which are precious as collection is usually difficult. Microfluidics can also be fully automated and the small dimensions of spatially separated microfluidic compartments allow the assembly of a multitude of individually controllable (cell culture or *vivo*) chambers on a single device. Together, these advantages facilitate high parallelization of experiments, high throughput of samples and reactions and thus improvement of reproducibility, fast analytical times and less contamination, and eliminating the need for skilled technicians. Finally, microfluidics provides a high throughput platform to irradiate samples in a contained, portable environment that can be easily integrated with additional cartridges upstream or downstream from the irradiation step. All these advantages allow a broad use of microfluidic technology in biomarker analysis, especially for dosimetry biomarkers widely used in the field of radiobiology.

However, microfluidics is still a complex technology and it can still take several years before being widely commercialized. Indeed, the difficulty to fully implement complex multi-step processes on a microfluidic cartridge is extensive and even simple benchtop methods, such as centrifugation, can become complicated on cartridge. Another concern, the choice of surface materials is crucial and currently several problems arise with non-specific binding leading to irreproducibility of result measurements and failure of devices [10].

It may be challenging to develop fully integrated microfluidic methods compared to automated benchtop ones, but after development is complete, the microfluidic method is likely more efficient economically and scientifically. Microfluidics and radiobiology must continue to develop side-by-side to realize the richness of the improvements these fields can gain from one another.

Acknowledgments

We thank the members of the Center for Applied Nanobioscience and Medicine for their participation in discussing some of the prior art.

The authors received financial support from the following sponsors: FZ was supported by the Center for High-Throughput Minimally-Invasive Radiation Biodosimetry (National Institute of Allergy and Infectious Diseases Grant U19 AI067773); JL received a fellowship from ARC (File No. SAE20140600948); SP received a 2015 scholarship from the Helios Education Foundation in Arizona.

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Fig. 1.

Complex cellular response to radiation. There are two kinds of effects due to ionizing radiation. On the one hand, direct effects create damages by affecting directly cellular components and molecules like cell membrane, mitochondria or DNA. On the other hand, ionizing radiations interact with water to create free radicals like ROS or RNS. These compounds could then initiate harmful chemical reactions within the cells and induce a variety of structural changes. Some enzymatic complexes (i.e. NADPH oxidase) have also been shown to be involved in ROS production and could be activated by radiation participating to indirect effects. Interestingly, cellular response to radiation is not limited to the irradiated cell itself but can also affect neighborhood cells. Thus, secreted cytokines after activation of some cellular pathways and ROS/RNS that diffuse by intracellular junctions may transmit an injured signal to the non-targeted cells. This phenomenon is known as the bystander effect. Finally, these signals can also diffuse far away from the irradiated cells by bloodstream and perturb organism as a whole: this is the abscopal effect. Abbreviations: ATM, ataxia telangiectasia mutated; IL1, interleukin-1; IL6, interleukin-6; IL8,

interleukin-8; MAPKs, mitogen-activated protein kinases; NF- κ B, nuclear factor-kappa B; NOX, NADPH oxidase; ROS/RNS, reactive oxygen species/reactive nitrogen species; TGF β , transforming growth factor beta; TNF α , tumor necrosis factor α ; TRAIL, tumor-necrosis-factor related apoptosis inducing ligand.

Fig. 2.

Current microfluidic approach to radiobiological studies. Microfluidics is currently used in three main domains in radiobiology: *in vitro* studies, *in vivo* studies and dosimetry biomarker studies. For each application, microfluidics leads to several advantages compared to classical models.

Table 1

Main studies using microfluidic devices to studying ionizing radiation effects.

Sample	Model	Application	Advantages	Disadvantages	Reference
Head and neck squamous cell carcinoma and rat liver	<i>In vitro</i>	To study radiation-induced cytotoxicity <i>ex vivo</i>	Maintains tissue in viable state by preserving 3D architecture; reproducible	Cells may react differently <i>in vivo</i> versus in the cartridge	[11]
Bone marrow	<i>In vivo</i> and <i>in vitro</i>	Bone marrow on-a-chip to use as model	Supports normal proportions of HSCs and progenitor cells and maintains spatial position within 3D-bone marrow niche; alternative to animal models; does not require exogenous cytokines	Time intensive – must be implanted in mouse for 8 weeks; not automated, requires scientist to implant device in mouse and insert eBM into microfluidic device	[12]
Fluorescent beads as models for cells	Technology prototype	Microbeam irradiator	Speed – goal of irradiation throughput of 100,000 cells per hour; allows for irradiation of non-adherent cells	Beam scattering through microfluidic device	[13]
<i>Caenorhabditis elegans</i>	<i>In vivo</i>	Tool for microbeam irradiation	Reduce used of anesthetics; microbeam irradiation of 16 nematodes in parallel	Beam scattering through microfluidic device	[14]
Blood	<i>In vitro</i>	Biodosimetry using qNPA	Point-of-care use; much faster assay, higher throughput, low cost; small amount of blood from finger-stick	Difficult to use whole blood in microfluidic device because it needs centrifugation	[15]
Cultured human lymphoblastoid cells	<i>In vitro</i>	Biodosimetry using γ -H2AX assay	Quick analysis of radiation dose; does not require skilled operator; could be adapted to test for other proteins	Expensive equipment needed (laser, spectrometer, syringe pump); potential clogging if cartridge is reused; must be used within a few days of radiation exposure	[16]