On-chip radiation biodosimetry with three-dimensional microtissues

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This paper reports an image-based, on-chip microtissue radiation biodosimeter that can simultaneously monitor radiation responses of multiple mammalian cell types. The microtissue chip is fabricated by molding molten agarose gel onto microfabricated patterns to form microwells, and seeding a variety of cell suspensions into different microwells inside the agarose gel. The camera of a mobile phone is used to collect images of an array of microtissues, and the color changes of microtissues upon X-ray irradiation allow accurate determination of cell death, which is related to radiation dose. The images can be transferred wirelessly, allowing the biodosimeter to be used for convenient and field deployable monitoring of radiation exposure.

Fast and accurate determination of radiation-induced damage to living cells, in a field setting, is highly desirable. Although a variety of radiation dosimeters including ion chambers, quartz fiber dosimeters, film dosimeters and polymer gel dosimeters can be used to detect the type and dose of radiation over a certain period of time, they cannot provide information on individual biological response to radiation. A biological dosimeter (i.e., biodosimeter) uses biomaterials such as cells to monitor radiation doses received by an individual and can provide important information on the bio-response of radiation exposure, which is highly complementary to physical dosimeters. Rapid biodosimetry in field settings can help to distinguish those who need urgent medical treatments after radiation injuries.1 The gold standard of biodosimetry is a dicentric assay that is often performed in peripheral blood lymphocytes to examine characteristic chromosome damage, but the dicentric assay takes a long time for microscopic scoring of a sufficient number of damaged cells, especially in the case of a low radiation dose where damaged cells are hard to find.2 Other techniques including micronucleus, translocation, premature chromosome condensation assays, and molecular biomarker methods (gamma-H2AX assay) have been proposed recently to detect radiation damage,3,4 but most of them require highly trained personnel and well-equipped facilities (i.e., microscope), and are invasive in nature. In addition, since different cells have different radiation susceptibility, it is imperative to derive radiation effects from multiple types of mammalian cells.5 But, existing biodosimetries have low throughput, are complicated, expensive, laborious and time-consuming when handling multiple cell types, and need to be carried out in centralized laboratory settings, which limit their use in field-deployable detection in places such as nuclear power plants, and medical or military facilities where continuous dose monitoring is necessary to ensure personal safety.

In vitro grown mammalian cells could be used to construct low-cost, facile, non-invasive, sensitive, potentially high-throughput and field-deployable biodosimeters. However, cells from two-dimensional (2D) monolayer cultures are too sensitive to environmental changes even in the absence of radiation, which make them unsuitable as responsive components in biodosimeters. Instead, cells in three-dimensional (3D) aggregates are in an environment close to physiological conditions and can mimic the tissue architectures and functions of human beings; meanwhile, these aggregated cells have shown higher stability than those from a 2D culture.6 Lastly, although many traditional techniques including spinning, hanging drop, microspheroid chips, and liquid-overlay methods have been used to make 3D microtissues, these techniques are laborious, and have low throughput and low uniformity in terms of density, shape and size.7–11 Photolithography based microfabrication techniques can generate size- and geometry-controlled microstructures, which can be used to make 3D microtissues with uniform size at high yield and low cost.12

This paper describes a new microtissue based on-chip biodosimeter that can accurately monitor the response of individuals to ionizing radiation in field settings and wirelessly transmit images to a remote location for analysis. The biodosimeter is based on two techniques: in vitro 3D microtissues and the high-resolution digital camera of a mobile phone to monitor cell death in real time for rapid determination of radiation exposure.15–18 An array of microtissues is exposed to X-ray radiation that causes cell death; after staining, dead cells can be identified from the color that is visible to the naked eyes. Images of the microtissues are taken with the camera of a mobile phone and transmitted wirelessly to a computer, where the color intensity of each microtissue is analyzed to determine the number of dead cells. The use of an array of micro-tissues greatly eliminates errors during measurements and allows multiple cell types to be used for studying biological response to radiation. This method has high sensitivity such that a low dose of ionizing radiation can be detected from a large area.

The micropost mold is fabricated using the reported photolithography technique.16 The depth and width of microposts are 1000 and
800 μm, respectively. In order to generate the 3D microtissue array, microwells are cast from the mold as shown in Fig. 1. A 2% low melting, molten agarose (85 °C) is poured on a tray and the micro-mold is placed on top until the agarose sets. The mold is carefully removed leaving microwells in the agarose film. HeLa cells (American Type Cell Culture, Manassas, VA) are cultured in 2D cell culture plates filled with RPMI 1640 media for 1–2 days until 80% confluence is achieved. The cells are detached from the substrate using 0.25% trypsin-EDTA and are counted using a hemocytometer after centrifugation at 1000 rpm for 5 min. In the next step, 2 × 10⁴ cells in media are seeded in a 9 × 9 array of microwells, where approximately 2.4 × 10³ cells occupy one well. The use of agarose as a substrate minimizes cell-substrate interaction, and the cell-to-cell adhesion drives spontaneous self-assembly of cells into 3D micro-tissues within an hour. Fig. 2A–D show the optical micrographs of 3D microtissues at various stages of formation taken at different times (1, 10, 30 and 60 min) after seeding HeLa cells into microwells. Most cells float in suspension just after seeding (1 min); about 50% and 80% of cells settled gravitationally in microwells after 10 and 30 min, respectively; almost all cells aggregate after 1 h of incubation. The initial cell aggregate becomes denser in 1–2 days, and the microtissue is stained using ethidium bromide (EB). Fig. 2E shows a fluorescence image of a single microtissue taken by using an Olympus BX-51M microscope under ultraviolet excitation, where the spherical shape of the microtissue can be seen clearly. In order to determine cell viability in the cultured microtissue, dual fluorescence staining is carried out with Calcein AM/EthD-1, where EthD-1 stains dead cells red by penetrating into membrane-damaged cells and combining with DNA in nuclei, and keeps live cells unstained, while Calcein AM stains live cells green leaving dead cells unaltered. The absence of red color indicates that cells are alive in microtissue. Due to the limited nutrition and oxygen available to cells inside microtissue, the speed of growth of microtissue is slower than that of the monolayer (doubling time of 24 h). But, cells in microtissue can stay alive in fresh culture medium for over 96 h outside the incubator, making microtissues very attractive for field-deployable radiation detection. One feasible way of having an even longer lifetime is to culture cells in the normal monolayer format, and form microtissue just a few hours before radiation detection.

![Fig. 1](image1.png) Fabrication of three dimensional microtissues in agarose gel.

![Fig. 2](image2.png) Optical images of formed microtissues taken at 1, 10, 30 and 60 min after seeding HeLa cells into microwells (A–D); fluorescence image of microtissue stained with EB (E) and Calcein AM/EthD-1 (F).

![Fig. 3](image3.png) Optical images of HeLa microtissues in microwells stained with trypan blue, not exposed to X-rays (A), exposed to X-rays for 1 h (B) where the inset picture shows microwells stained with EthD-1 after X-ray exposure; the intensity of blue color from trypan blue measured at different times after 1 h X-ray irradiation of microtissues containing different cell lines (C); the corresponding survival fraction of the cell lines obtained from the MTT assay (D). Error bar represents the standard error of 5 independent experiments.
A Mini-X X-ray tube (Amptek, Inc., Bedford, MA) operating at a tube voltage of 40 kV and a current of 100 μA is used to generate primary X-rays. The surface dose rate at a distance of 5 cm from the tube is measured using a handheld radiation dosimeter (Mirion RAD-60, Freshwater Systems, Greenville, SC) and is found to be 0.4 Gy h⁻¹. The 9 × 9 array (surface area of ~1 cm²) of microwells loaded with HeLa cells is then fixed at a distance of 5 cm from the X-ray source, which is fitted with a collimator resulting in an output cone angle of 10° for the emerging beam. The X-ray beam covers an area of ~2.4 cm² on the exposed surface that is sufficient to uniformly irradiate the entire array. After X-ray irradiation for different times ranging from 1 min to 1 h, trypan blue, a non-fluorescent dye that stains dead cells blue while leaving live cells unaltered, is added into microwells to stain cells in the microtissue. Fig. 3A shows a photograph of microwells taken with the camera (5 MP resolution) of an iPhone 4, where HeLa cells are stained with trypan blue after formation of microtissue. The absence of blue color shows that most of the cells in microtissue are alive. Fig. 3B shows the image of 1 h X-ray irradiated microtissues that have been seeded with the same number of HeLa cells followed by trypan blue staining. The blue color of microtissues in each microwell is clearly visible to the naked eye and the color intensity of image is proportional to the number of dead cells. The inset picture in Fig. 3B shows the fluorescence image of cells, after EthD-1 staining, taken with a phone camera under an UV light, where dead cells are in red.

Cell viability is monitored in real time for dosimeters containing HeLa cells (human cervical cancer cell), MG-63 (human osteosarcoma cell), and LNCaP cell (human prostate adenocarcinoma cell), respectively. This is done by measuring the color intensity of each microwell from images taken every 30 min following a 1 h X-ray exposure (0.4 Gy). The images are analyzed with Image Pro Plus 6.0 (Media Cybernetics, Bethesda, MD), which can correct the background and allow us to obtain the intensity of an area of interest. Fig. 3C shows that the intensity of blue color increases and saturates to a value of ~100 after 9 h; under the same conditions, it takes 11 h and 14 h for MG-63 and LNCaP cells, to reach the same color intensity, respectively. To correlate the intensity of trypan blue with cell viability, an MTT assay is performed on the three cell lines at 30 min intervals after exposure to 0.4 Gy X-rays. The MTT assay can detect live or dead cells by measuring the integrity of the cell membrane. Following a 1 h X-ray irradiation, ~30% HeLa cells are dead after 9 h (Fig. 3D). However, ~28% MG-63 and ~33% LNCaP cells die after 11 h and 14 h, respectively. Results confirm that different cell lines respond differently towards the same X-ray dose with HeLa and LNCaP cells being the most and the least sensitive to X-rays, respectively. This can be explained in terms of the difference in the cell cycle: HeLa cells double every 24 h, whereas MG-63 and LNCaP cells double every 26 h and 60 h, respectively. After X-ray induced DNA damage, cells will not die until they are in the mitotic

Fig. 4 An optical image showing controlled cell seeding in microwells (A), where the top row contains HeLa, MG-63 and LNCaP cells (from left to right), and the bottom row has no cells; HeLa cell viability against the color intensity of trypan blue stain, the inset shows the variation of color intensity for trypan blue stain with X-ray dose (B); percentage of dead cells for three different cell types after exposure to different X-ray doses (C); color intensity of trypan blue stained HeLa microtissues for continuous and cumulative X-ray exposures (D). Error bar is the standard error of 5 independent experiments.

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period where damaged DNA fails to duplicate. Thus, quantifying the blue color of trypan blue stained microtissues over time allows in situ real time monitoring of radiation responses.

High throughput radiation detection and monitoring is achieved by introducing three cell lines (HeLa, MG-63, and LNCaP) into a single biodosimeter. By controlled pipetting of 1 μl of cell suspension into each microwell using a small volume tip, an array of different microtissues is made on the same biodosimeter for high throughput applications. Fig. 4A is an optical micrograph of an array of microtissues, where three microwells in the top row contain HeLa, MG-63 and LNCaP cells (left to right), and three empty wells in the bottom row are controls. The compact arrangement allows all cells to be irradiated simultaneously with a uniform X-ray dose. After irradiating for varying times, cells in microwells are stained with trypan blue followed by imaging with a phone camera. Fig. 4B shows that the increase in intensity of the blue color is proportional to the decrease in viability of HeLa microtissues. Similar results are obtained for MG-63 and LNCaP microtissues. The linear relation between color intensity and X-ray dose is also plotted accordingly.

The increase in intensity of the blue color is proportional to the decrease in viability of HeLa microtissues. Similar results are obtained for MG-63 and LNCaP microtissues. The linear relation between color intensity and X-ray dose is also plotted accordingly. The current detection limit of our biodosimeter for HeLa microtissues is estimated to be ~5.0 mGy from the calibration curve. For microtissues of MG-63 and LNCaP cell lines the detection limits are estimated to be ~8.0 and 9.5 mGy, respectively. The limit of detection also depends on the type of cell, because some cells are more sensitive to radiation than others.

Fig. 4C shows cell death at different X-ray irradiation doses for three different types of microtissues where the percentage of dead cells directly correlates with the intensity of blue color from trypan blue stain. At higher doses more cells die, resulting in a more intense blue color. In contrast to conventional radiation dosimeters, microtissue biodosimeters can directly monitor the effect of both cumulative exposure and continuous radiation exposures on mammalian microtissues. In the case of cumulative exposure, the total dose is fractionated to 1 h day^{-1} (0.4 Gy day^{-1}) for 8 days, whereas for continuous exposure the whole dose (3.2 Gy) is applied continuously for 8 h in one fraction. Fig. 4D shows the continuous irradiations kill more cells in the HeLa microtissue than the cumulative irradiations, especially when the irradiation time is longer than 3 h. The difference in cumulative and continuous exposures is mainly caused by self-repairing of small amounts of DNA damage. Cumulative exposure provides time for the damaged DNA to self-repair between exposures while in the case of continuous exposure the damage accumulates faster than it can be repaired.

Conclusions

An image-based on-chip radiation biodosimeter using three dimensional microtissues has been built and used to detect cellular response of continuous and cumulative ionizing radiations. The three dimensional microtissues of multiple cell types, with defined structures and dimensions, can be fabricated at high yield and low cost, and can be conveniently imaged with the camera of a mobile phone. The color changes of microtissues upon exposure to ionizing radiations are detected and quantified, which allows facile and rapid transmission of radiation information to determine responses of individuals in field settings.

Notes and references

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