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Supporting information

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Three-dimensional microtissues as in vitro model for personalized radiation therapy

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Radiation can destabilize microtubule network in cells, which can further affect cell migration. Figure A1 and A2 shows the extensive microtubule network of untreated cells. This phenomenon correlates with a healthy cell where cargo-carrying motor proteins used the microtubule network to move essential proteins intracellularly.[43] In other experiments, cells were treated with 20GY X-ray irradiation. Punctate structures form in some cells with 20GY of X-ray radiation (Figure B1 and B2), disassembly of microtubule network in most cells can be seen at 20GY (Figure B1 and B2). There are less red and green microtubes with increased X-ray irradiation. The florescence integrated intensity of sample A1, A2 and sample B1, B2 is showed in figure 1C, where red bar represents α-tubulin, while green bar represents vinculin using ImageJ to calculate.

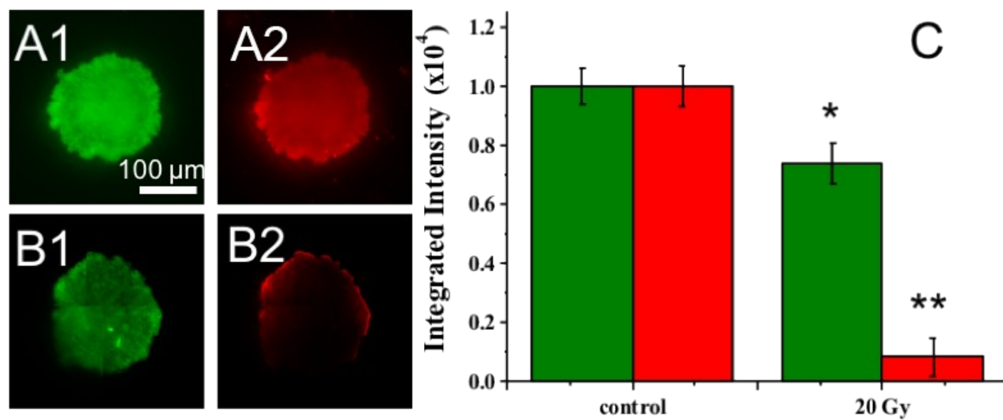

Figure 1: Fluorescent images of microtubules in cells without X-ray exposure (A1 and A2). Fluorescent images of microtubules in cells with 20Gy (B1 and B2). The integrated intensity of florescence of two samples (C).

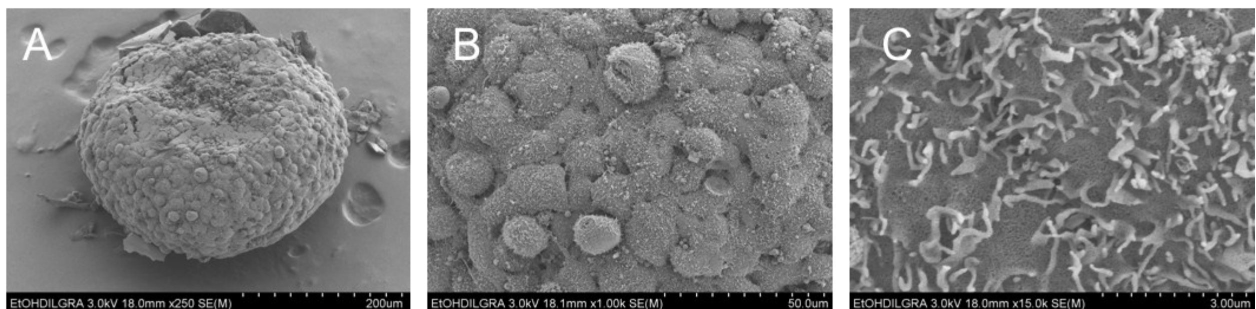

Figure 2: SEM image of 3D microtissue (A)-(C). The scale bar is 200, 50, and 3 µm, respectively.

In order to determine cell viability in the cultured microtissue, fluorescence staining is carried out with 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, Figure 3A shows a fluorescence image of a microtissue with 3Gy using a z-stack. A fluorescence image of a microtissue with 15Gy using a z-stack is showed in Figure 3B. There are less cells are killed in the core than the cells near the surface of the microtissue after X-ray irradiation. The absence of red color indicates that cells are alive in microtissue. The integrated intensity of florescence of two samples is showed in Figure 3C.

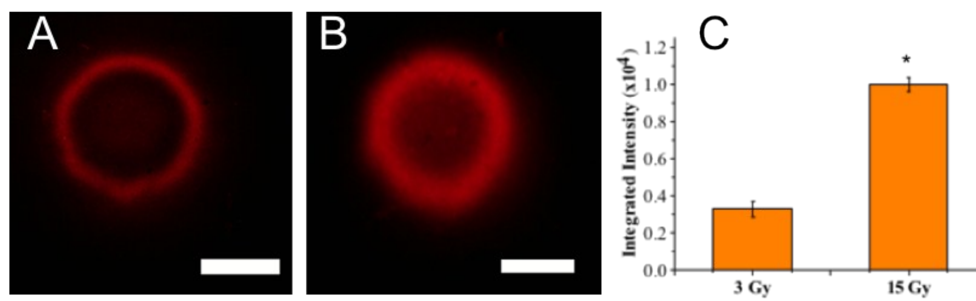

Figure 3: Fluorescent images of 3D microtissue with 3Gy stained with EthD-1 (A), with 15Gy (B). The integrated intensity of florescence of two samples (C). Scale bar is 200μm.

Reference

43. C. Yates, Advances in Cancer Research, 2007, **97**, 225–246.