Enhanced Radiation Therapy with Gold Nanoparticle-Loaded Multilayer Microdisks

Autumn D Paro[1], Peipei Zhang[1], Yong Qiao[1], Junifei Xia[2], Jingjiao Guan[2], Liyuan Ma[1], Ming Su[1].
[1]Department of Chemical Engineering, Northeastern University, Boston, MA 02115.
[2]Department of Chemical and Biomedical Engineering, Florida State University, Tallahassee, FL 32310.

Statement of Purpose: Radiation therapy with ionizing X-ray is an important component of cancer treatments[1]. Recent advances in physics, biology and clinical aspects have considerably improved accuracy and efficiency of dose delivery to targeted tissue. However, the challenge of delivering radiation accurately to malignant tissues while minimizing damage to normal tissue, remains. A variety of X-ray radiation techniques have been used to minimize dose on normal cells or maximize dose on cancer cells[2]. Dose can be fractionated over time to allow normal cells to recover, while cancer cells that are relatively radioresistive in the first treatment move into a relatively radiosensitive phase of cell cycle in the next treatment; the dose can be fractionated over space to intersect at tumors from several directions to spare normal cells along beam path. Image-guided radiation therapy and intensity-modulated radiation therapy can be used to maximize X-ray doses on tumors and conform to tumor's 3D shapes with beams of different intensities. Alternative methods can be used to improve discrimination between tumor and normal tissues. Radio-protective drugs have been used as free radical scavengers to protect normal cells from damage[3]. Radiosensitizers such as oxygen, oxygen carrying blood substitutes and radiosensitive drugs have been used to enhance the effects of given X-ray doses. Nanoparticles of heavy elements such as gold and platinum have been studied with the intention to enhance X-ray contrasts between normal and cancer cells. A challenge is that tumor killing effects in the presence of nanoparticles are two orders of magnitude smaller than predicted effects. If hundreds of thousands nanoparticles could be placed in vicinity of cells, a large amount free radicals will be available for DNA damage, and the total X-ray dose can be reduced to receive the same treatment effect.

This project describes a method to deliver a large amount of radio-sensitizing nanoparticles to tumor cells by packing nanoparticles into polyelectrolyte microdisks that attach on surfaces of cancer cells. Microdisks are made by integrating microcontact printing and layer-by-layer assembly; and each microdisk contains over 10^5 gold nanoparticles. These microdisks can be released by dissolving PVA film in water, and attached onto cell surface via electrostatic interaction. Upon exposure to X-ray, a higher level of DNA damage can be induced in cells. The enhanced DNA damage in the presence of X-ray radiation and nanoparticle-loaded microdisks has been confirmed with single cell array based halo assay, express of DNA repair proteins, as well as micronucleus assay.

Methods: Nanoparticle-loaded microdisks were printed on glass substrate to form an ordered array. Cells were then attached to microdisks through electrostatic interactions, exposed to X-ray radiation for 10 min and collected by washing off microdisks. The viability of cancer cells (A 172) attached on an array of microdisks was tested using HaloChip assay, micronucleus assay and expression of DNA repair protein.

Results: HaloChip assay is used to assess DNA damage induced by microdisks and X-ray radiation at the single cell level as follows. Nanoparticle-loaded microdisks are printed on glass to form an ordered array. Cells are attached to microdisks and embedded in an agarose gel. After gel solidification, sample is immersed in an aqueous solution of NaOH for lysis. Damaged DNA fragments self-diffuse into gel matrix and are stained with ethidium bromide, forming a diffusive ring around each nucleus. Cells attached on microdisks without nanoparticles have less damage than those on microdisks with nanoparticles. Cells attached onto microdisks with 3 and 4 layers of nanoparticles show similar damage. DNA damage has also been assessed with expression of a DNA repair protein, γ-H2AX, which is recruited to repair DNA double strand breaks. Cells were exposed to X-ray radiation for 10 min and collected. Primary and fluorescent labeled secondary antibodies were added sequentially in cell culture medium. The secondary antibodies are conjugated with fluorescein, which allows quantification of γ-H2AX expression by measuring fluorescence intensity. Cells irradiated on microdisks without nanoparticles show weak green fluorescence, indicating a small amount of γ-H2AX. Cells irradiated on microdisks with nanoparticles show strong γ-H2AX expression. Radiation damage to nuclei have been assessed with micro-nucleus assay. Cell nuclei are stained with DAPI. Cells irradiated on nanoparticle-loaded microdisks have less micronuclei than those irritated on microdisks with nanoparticles. The number of micronuclei per 2,000 cells increases with the layer of nanoparticles in microdisks. No significant difference exists between cells radiated on microdisks with three and four layers of nanoparticles.

Conclusions: This has proved the concept of using microdisks that contain gold nanoparticles to enhance X-ray radiation killing of cancer cells. HaloChip assay has confirmed that nanoparticle-loaded microdisks can cause more DNA damage upon X-ray radiation. The increased fluorescence signal of DNA repair protein (γ-H2AX) indicates that more double strand break in DNA of X-ray irradiated cells. Micro-nucleus assay indicates the genome level damage of cells. Cells irradiated on nanoparticle-loaded microdisks have shown less micronuclei than those irritated on microdisks with nanoparticles. More cells can be killed due to combined effect of X-ray radiation and nanoparticles.

References:

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