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Multicellular dosimetric chain for molecular radiotherapy exemplified with dose simulations on 3D cell spheroids

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ABSTRACT

Purpose: Absorbed radiation dose-response relationships are not clear in molecular radiotherapy (MRT). Here, we propose a voxel-based dose calculation system for multicellular dosimetry in MRT. We applied confocal microscope images of a spherical cell aggregate i.e. a spheroid, to examine the computation of dose distribution within a tissue from the distribution of radiopharmaceuticals.

Methods: A confocal microscope Z-stack of a human hepatocellular carcinoma HepG2 spheroid was segmented using a support-vector machine algorithm and a watershed function. Heterogeneity in activity uptake was simulated by selecting a varying amount of the cell nuclei to contain ^{111}In, ^{125}I, or ^{177}Lu. Absorbed dose simulations were carried out using vxlPen, a software application based on the Monte Carlo code PENELOPE.

Results: We developed a schema for radiopharmaceutical dosimetry. The schema utilizes a partially supervised segmentation method for cell-level image data together with a novel main program for voxel-based radiation dose simulations. We observed that for ^{177}Lu, radiation cross-fire enabled full dose coverage even if the radiopharmaceutical had accumulated to only 60% of the spheroid cells. This effect was not found with ^{111}In and ^{125}I. Using these Auger/internal conversion electron emitters seemed to guarantee that only the cells with a high enough activity uptake will accumulate a lethal amount of dose, while neighboring cells are spared.

Conclusions: We computed absorbed radiation dose distributions in a 3D-cultured cell spheroid with a novel multicellular dosimetric chain. Combined with pharmacological studies in different tissue models, our cell-level dosimetric calculation method can clarify dose-response relationships for radiopharmaceuticals used in MRT.

1. Introduction

Molecular radiotherapy (MRT) makes use of different cell mechanisms (e.g. receptor binding) to deliver lethal radiation doses selectively to malignant cells. Despite the large number of treated patients and successful clinical trials over the past decades, considerable uncertainties still remain regarding the optimization of MRT [1,2]. The dosimetry of MRT is still developing, and the key issue to establish is how the administered activity, the absorbed radiation dose distribution, and the clinical response and side effects relate to each other, and how this knowledge can be used to plan more individualized treatments.

For internally administered radionuclides, absorbed radiation dose calculations have long based on scintigraphy studies combined with the medical internal radiation dose (MIRD) formalism on a macroscopic level [3,4]. Applying the MIRD formalism on a microscopic level has also been studied [5]. The cell-scale morphology of tumors (cell dimensions and tissue packing) shows variation both between patients and within a patient. Relatively long irradiation ranges can balance effects of small-scale non-uniformities in the activity distribution. However, for short-range radiation, the absorbed radiation dose distribution can contain steep gradients in accordance with the non-homogeneous distribution of the radiopharmaceutical between cell surfaces, cytoplasm, and nuclei

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depending on the cell type. For example, in the case of peptide receptor radionuclide therapy (PRRT), it has been shown that using a different chelator, peptide and/or radionuclide can affect strongly the cellular binding affinities of the radiopharmaceutical. This in turn results in differing uptake and dose distribution in the malignant and normal tissues.

Studying internal dosimetry at biologically relevant scales appears to be essential for the development of MRT [6]. Therefore, utilizing *in vivo* mimicking tumor cell cultures is valuable. *In vitro* three-dimensionally (3D-)cultured tumor cell aggregates, also known as spheroids, resemble their *in vivo* counterparts more closely than conventional two-dimensional (2D) cell culture systems; the cells cultured in 3D retain their natural architecture and functionality better than those cultured in 2D. Various culturing techniques have been exploited to create 3D tumor models to study tumor biology, pathogenesis as well as the effect of anti-cancer compounds [7], including radiopharmaceuticals used in MRT [8,9].

3D cell cluster computational models have frequently been based on simple geometries like nested spheres, cylinders, and cubes mimicking cells and nuclei [5,10]. However, the computational capacity and imaging methods that are accessible today enable more sophisticated cell-level dosimetric modeling. A 3D-cultured tumor can provide detailed morphological data as a starting point for the MRT dosimetric chain. The cellular structures such as the nucleus, cytoskeleton, and plasma membrane can be visualized using fluorescent labels and confocal microscopy. Furthermore, the cell-level distribution of radiopharmaceuticals can be studied by labeling [11].

To perform dose calculations, the imaging data needs to be segmented. Manual cell tracking is labor-intensive and subjective, and therefore automated segmentation algorithms are needed. Intensity thresholding is frequently used in the segmentation of microscopy images [12]; however, thresholding cannot be applied to multichannel data and cells (or other structures) that touch each other and are inseparable. Watershed methods [13] are often used to segment touching objects, but they tend to lead to oversegmentation. To overcome these problems, new ways to classify and regularize image data for segmentation algorithms are needed. A support-vector machine (SVM) [14] can be efficiently taught to classify structures from 3D confocal microscope data by giving it a set of training voxels and their classification. In this study, we utilized such an SVM classifier and morphological preprocessing to avoid oversegmentation in combination with a watershed transform to create a segmentation process which has potential to be fully automated with more datasets.

Here, we present a new multicellular dosimetric chain (see the schematic Fig. 1) that combines confocal microscope imaging data of a 3D-cultured tumor cell spheroid, an automated segmentation algorithm, and Monte Carlo (MC) dosimetric calculations to assess the absorbed radiation dose distribution down to the cell-scale (micrometer scale). To exemplify, we simulate the behavior of activity vs. dose distributions in the case of the Auger electron and internal conversion (IC) electron emitters ^{125}I and ^{111}In, and the low-energy β electron emitter ^{177}Lu, all of which have very distinct spectra of the emitted electron and photon radiation [15]. ^{125}I is a radionuclide that has been studied widely regarding multicellular dosimetry [9] and it can be considered as a benchmark ("a model Auger emitter") although its clinical applications for MRT have been limited. On the other hand, ^{177}Lu is actively used today for MRT in PRRT, and also in e.g. ^{177}Lu-PSMA therapy. Before ^{177}Lu, ^{111}In was used for PRRT, with less efficient results; a fact that makes the comparison of these two radionuclides intriguing. Performing dose calculations with MC methods can be time-consuming, and more effective ways, such as utilizing dose point kernels (DPKs), have often been preferred. To corroborate our MC dosimetric calculations, we also produced DPKs and compared them to published results.

2. Materials and methods

2.1. Tumor spheroid culture and imaging

Human hepatocellular carcinoma HepG2 (HB-8065, ATCC) cells [16] were maintained in 75 cm^2 culture flasks in DMEM (31966-021, Gibco) supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin. During the maintenance the cells were subcultured twice a week in a ratio of 1:5.

The cells were transferred to 3D culture environments by embedding them in GrowDex®, a nanofibrillar cellulose hydrogel, as previously described by us [17,18]. Cultures were formed in an 8-well Chamber Slide system (177445, Nunc). Thereafter, the cells were detached with 0.25% trypsin-EDTA (25200-072, Gibco) and the cell suspension was mixed with the hydrogel and culture medium to obtain a 1.0–1.2 wt% hydrogel concentration and a cell density of 0.8 × 10^6 cells/ml. Lastly, a culture medium was added

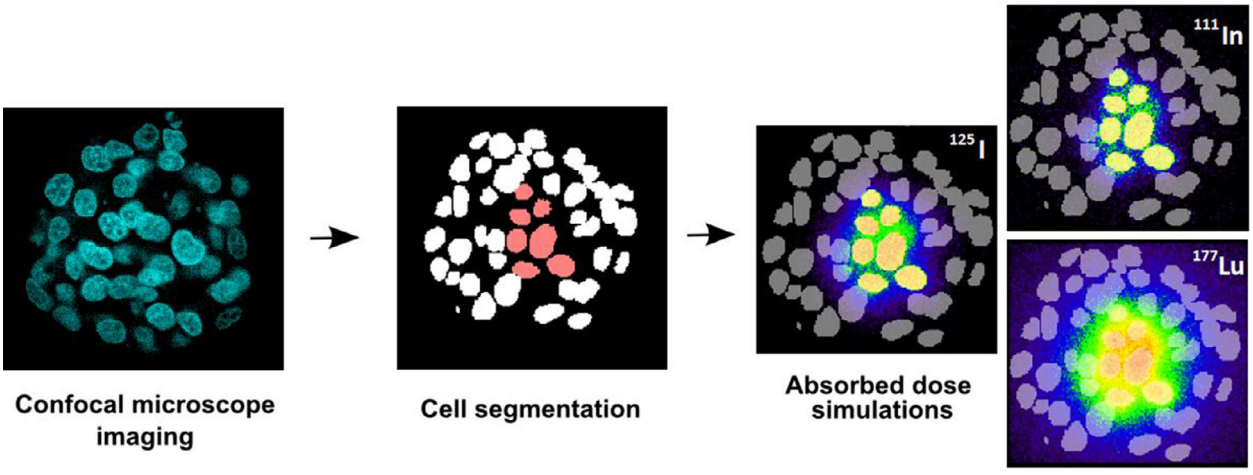

Fig. 1. Multicellular dosimetric chain: a confocal microscope image of a cell spheroid was segmented and used as a basis for dosimetric calculations. On the left, the Hoechst stained cell nuclei are shown in cyan in a confocal microscope image slice. The 20 innermost cell nuclei were filled with a uniform activity (shown in red in the segmented image slice in the middle), and the resulting absorbed radiation dose distributions are shown on the right (the colour scale in the images is schematic).

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