Innovative method of superimposing Gamma-CBCT images with live confocal imaging to assess Cellular Responses to Radiation

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Abstract

Background: Understanding the real-time effects of radiation on live cells remains a challenge due to limitations in current imaging and targeting techniques. Traditional methods like clonogenic assays and fixed-cell imaging fail to capture dynamic and spatially-resolved cellular responses.

Objective: This study introduces and evaluates three novel phantom-based methodologies designed to visualize and analyze radiation-induced changes in live cells with high spatial precision.

Methods: All three techniques employed confocal imaging dishes embedded in brain-mimicking agarose gel phantoms. Live cells were cultured on treated glass-bottom dishes and exposed to targeted gamma radiation. Technique 1 involved direct embedding of the dish without any positional markers. Technique 2 incorporated a small metal grid and aperture film to partially localize radiation effects. Technique 3 employed a full-scale metal grid for precise spatial mapping and dose-effect correlation. Cone-beam CT (CBCT) was used for localization, and post-radiation cellular effects were assessed using confocal microscopy.

Results: Technique 1 allowed basic observation of radiation effects but lacked spatial localization. Technique 2 enabled partial localization via CBCT, yet limitations in grid size and imaging overlay reduced resolution. Technique 3 demonstrated superior precision, enabling clear mapping of dose-dependent cellular responses in individual grid sections. However, challenges such as metal-media interaction and grid handling remained.

Conclusions: The full-scale metal grid approach (Technique 3) offers a powerful platform for studying real-time radiation effects on live cells with high spatial resolution. These findings open new avenues in radiobiology, particularly in optimizing radiotherapy protocols and investigating radiation damage mechanisms. Further refinements, including grid material optimization and integration with advanced imaging modalities, are warranted.

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Keywords:
Radiation biology, live cell imaging, confocal microscopy, phantom model, metal grid, dose mapping, cone-beam CT, radiotherapy

Introduction

Radiation exposure impacts cellular structures and functions significantly, leading to DNA damage, apoptosis, and other critical effects (Durante & Loeffler, 2010). Despite extensive research, a major limitation persists in directly observing these effects on live cells due to the lack of precise methodologies.

Traditional approaches such as clonogenic assays and fixed cell imaging provide indirect or static information, failing to capture real-time dynamics and spatial distribution of radiation-induced cellular effects (Franken et al., 2006). The limitations of these methods underscore the need for innovative techniques that integrate radiation delivery systems with advanced imaging modalities like confocal microscopy. This integration can enhance our understanding of dose-dependent cellular responses and support the development of more targeted radiotherapy protocols (Van Gysen et al., 2020).

As of now there is no such study to see the live cell changes after the radiation. In this study, we introduce three novel approaches to assess radiation effects on live cells. These methods employ customized phantoms mimicking brain tissue, confocal dishes, and metal grids to precisely correlate radiation exposure with cellular outcomes. Our findings demonstrate the feasibility of these techniques and their potential applications in radiobiology.

Methodology

To study the spatial and biological effects of targeted radiation on live cells, two phantom-based techniques were developed using confocal imaging dishes embedded in brain-mimicking agarose gel. In both approaches, 20 mm glass-bottom confocal dishes with treated surfaces were selected to ensure optimal cell adhesion and imaging compatibility. Live cells were cultured according to standard protocols, seeded onto the dish, and allowed to adhere for 12–24 hours under appropriate conditions. A 2% agarose gel solution was prepared to mimic brain tissue and poured into molds to solidify. A cavity matching the dimensions of the confocal dish was then carefully cut into the gel to securely embed the dish. In the first technique, the dish containing adherent live cells was placed directly into this cavity, and the phantom was positioned inside a Gamma Knife machine. Cone-beam CT (CBCT) imaging was used to locate the dish within the phantom, after which radiation was delivered to the targeted region. Post-exposure, the dish was removed and analyzed using confocal microscopy to observe radiation-induced cellular effects. In the second technique, spatial precision was further enhanced by incorporating a small metal grid at the bottom of the confocal dish as a radiographic and positional reference. Additionally, a thin film with a precisely cut circular aperture was aligned over the dish to guide radiation targeting. The dish, with the metal grid and alignment film, was embedded in the phantom using the same cavity-cutting method. CBCT imaging facilitated accurate localization of the grid, and radiation was delivered precisely through the film aperture. Following exposure, confocal microscopy was used to assess the spatial impact of radiation, particularly around the grid region, providing higher-resolution insights into localized radiation effects on the cultured cells.

Technique 3: Full-Scale Metal Grid

This technique involves the use of a customized full-scale metal grid placed within a confocal imaging dish to allow precise radiation targeting and post-irradiation imaging of cultured cells. The process begins with the selection of a confocal dish featuring a 20 mm glass bottom and a treated surface to ensure optimal cell attachment. It is essential to verify the optical clarity and imaging compatibility of the dish. The metal grid is then customized by cutting it to a size slightly smaller than the diameter of the dish using precision tools. The grid should be designed with uniformly spaced squares or rectangles to facilitate easy spatial mapping during imaging and radiation delivery. Both the grid and any spacers are sterilized using autoclaving or a 70% ethanol rinse to prevent contamination.

Following sterilization, the grid is placed centrally and leveled within the dish. Care must be taken to ensure the grid does not tilt or make contact with the glass bottom, as this could interfere with imaging. Moldable materials such as dental wax are used to align the grid accurately and can be shaped as needed to target specific zones for radiation exposure. Once the grid is positioned, live cells or tissues are cultured and prepared following standard laboratory protocols. Ensuring cell viability and uniformity is critical before seeding. Cells are then carefully seeded onto the treated glass bottom, and allowed to attach over a period of 12–24 hours. Culture media is added in sufficient volume to submerge the cells without touching the grid, ensuring the metal remains above

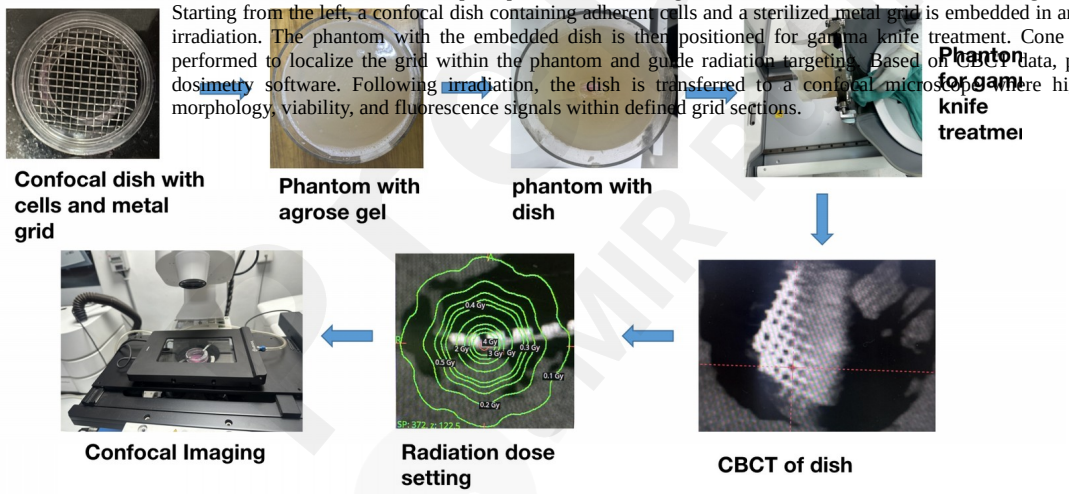
the liquid interface.

After cell attachment, the entire dish setup is embedded into an agarose gel phantom using the same procedure described in Technique 1, thereby creating a stable and reproducible environment for radiation studies. For grid localization, the embedded dish is secured in a CT scanner to prevent movement and maintain sterility. A high-resolution CT scan is then performed to visualize the exact position and alignment of the metal grid within the phantom. Based on these scans, dosimetry software is employed to calculate the precise radiation dose required for each grid section, factoring in cell type, radiation sensitivity, and experimental goals.

For radiation delivery, the confocal dish is carefully aligned within the irradiation setup to match the planned grid coordinates. Radiation doses are then administered to specific grid regions while maintaining sterile conditions throughout. Post-irradiation, the culture media is refreshed to preserve cell viability. The grid and dish are inspected for any physical distortions or contamination, and any debris is removed to prepare the sample for imaging.

For confocal imaging, the dish is transferred to the microscope under sterile conditions. The confocal microscope is calibrated to focus on the grid coordinates, and molded or labeled sections (e.g., A1, A2, B1, B2) are identified for imaging. Magnification and fluorescence settings are adjusted appropriately, and high-resolution images are captured from each grid section to assess cellular responses. Observations typically include cell morphology, viability, and fluorescence-based markers. Finally, comparative analysis of images across different grid sections is conducted to determine dose-dependent effects and correlate them with the spatial pattern of radiation exposure.

Figure: Workflow of Full-Scale Metal Grid-Based Targeted Radiation on Cultured Cells
This schematic illustrates the complete process of delivering localized radiation to cultured cells using a confocal dish integrated with a full-scale m
Starting from the left, a confocal dish containing adherent cells and a sterilized metal grid is embedded in an agarose gel phantom to stabilize it for ima
irradiation. The phantom with the embedded dish is then positioned for gamma knife treatment. Cone Beam Computed Tomography (CBCT) in
performed to localize the grid within the phantom and guide radiation targeting. Based on CBCT data, precise radiation dose planning is carried
dosimetry software. Following irradiation, the dish is transferred to a confocal microscope where high-resolution imaging is conducted to as
morphology, viability, and fluorescence signals within defined grid sections.



Data Analysis

1. Image Processing

1. Use image analysis software (e.g., ImageJ or MATLAB) to quantify parameters such as cell density, nuclear integrity, and fluorescence intensity.
2. Normalize data to control areas for accurate comparison.

2. Statistical Analysis

1. Perform statistical tests to validate results and ensure reproducibility.
2. Use software tools such as SPSS for detailed analysis.

3. Result Interpretation

1. Interpret data to draw conclusions on the dose-response relationship.

2. Identify trends and outliers for further investigation.

Results

The study of radiation effects on live cells necessitates precise localization and dose-effect correlation to understand cellular responses accurately. Three techniques were explored to achieve this goal, each building upon the limitations of the previous one.
In the first technique, direct radiation exposure was achieved successfully; however, the exact location of the dose could not be determined using the confocal microscope. This limitation made it impossible to establish a clear correlation between the dose and its effect on cells, thus hindering the experimental analysis.To address these challenges, a small metal grid was introduced in the second technique. This grid enabled partial localization of the radiation dose using Cone Beam Computed Tomography (CBCT) and confocal imaging. While this approach improved spatial identification compared to the first technique, its small size restricted the visualization of high-dose radiation effects. Furthermore, the process of superimposing images to map the dose-effect relationship proved to be challenging, limiting the method's overall efficacy. The third technique introduced a large metal grid, which resolved the shortcomings of the earlier approaches. This grid allowed precise localization of radiation effects for each labeled grid box, enabling confocal imaging to clearly display specific dose effects on cells. By overcoming the limitations of inadequate localization and poor mapping, this technique provided reliable spatial resolution and dose-effect correlation, making it the most robust method of the three.In conclusion, the evolution of techniques highlights the importance of refining tools and methodologies for studying radiation effects on live cells. While Techniques 1 and 2 offered incremental improvements, Technique 3 emerged as the most effective approach, providing the accuracy and clarity needed for high-resolution radiation studies.

Discussion

The evolution of methodologies for studying radiation effects on live cells has been highlighted through this study. The first technique provided a foundational framework but lacked spatial precision, making it challenging to correlate radiation dose with cellular responses.
In contrast, the second technique introduced a small metal grid, enabling partial spatial localization. However, its limited size restricted comprehensive analysis, particularly at lower radiation doses. Moreover, challenges in superimposing CBCT and confocal images remained unresolved.
The third technique emerged as the most effective approach, integrating a full-scale grid with labeled regions. This innovation allowed precise localization of radiation effects and detailed dose-effect mapping using confocal microscopy. Despite its success, the interaction of the metal grid with the cell culture media poses a limitation. This issue could alter cellular behavior and potentially compromise experimental reproducibility (Coleman & Blakely, 2017).
Future studies should focus on addressing these challenges by exploring grid coatings or alternative materials. Additionally, the integration of advanced imaging techniques such as multiphoton microscopy could further enhance the spatial and temporal resolution of this methodology.
These findings open new avenues for real-time studies on radiation biology. This methodology can support research into radiation-induced cellular damage and its mitigation strategies, with implications for radiotherapy optimization and radiation safety.

Conclusion

The developed methodology represents a significant advancement in studying the effects of radiation on live cells. The third technique, with its grid-based approach, provides precise spatial and dose-

effect correlation, although challenges like media interaction require attention. This work paves the way for future studies focusing on refining these methods and exploring their potential in broader applications.

Limitations

1. Interaction of the metal grid with culture media, potentially affecting cell viability or behavior.
2. Risk of grid displacement during handling, affecting reproducibility.
3. Technical challenges in fabricating grids with consistent quality.

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