RACE
RAdiation resistance of cancer CElls using Geant4 DNA

Sept 2013 – Sept 2015

Coordinators: L. Maigne, E. Miot-Noirault
Scientific skills and synergies

- Radiobiology
- Radiochemistry
- Microscopy
- 3D cell culture
- Geant4-DNA
- Preclinical imaging
- Geant4
- Geant4-DNA
- Software development / Computing
- Dosimetry
- Treatment planning
- Preclinical studies
- Vectorization of dedicated molecules

GDRMI2B - Nano Workshop - CENBG - 28/03/14
How to improve resistant cancer treatments?

- Develop specific vectorized NP or molecules
- melanoma and chondrosarcoma spheroids

- Develop tools for multi-scale approach
  - Monte Carlo simulation
  - Cell population modeling

Prediction of biological damages
Milestones

3D cell cultures
Melanoma + chondrosarcoma

Gadolinium NP

3D imaging

X Ray radiation

Clonogenicity
γH2AX foci

Cell population modeling

Beam delivery modeling

Interactions with Gd NP
Tracking of secondary particles
DNA fiber modeling
DNA damages

Dedicated software
Geant4/GATE
Low Energy models
Geant4 DNA

in vitro experiments
in silico modeling
CELL POPULATION
Why 3D cell models?

- Human model
- Low sensitivity to ionizing radiation
- Ability to vectorized NP *in vivo* to increase DNA damages

*From Hirschhaeuser et al., 2010, J Biotech*
3D cell culture

Selection criteria

- Clonogenic ability
- Ability to form multicellular spheroids in methyl-cellulose containing medium

Dissociated Cells

V-shaped microwells
Plastic untreated for culture

37°C

Melanoma

- SkMel 28
  Clonogenicity 17%
  Day 6
  \( \varnothing 570 \, \mu m \)

Chondrosarcoma

- SW1353 or HEMC-SS?
  Clonogenicity?
  Day 7 (\( \varnothing \pm 500 \, \mu m \))

Day 7 (\( \varnothing \pm 500 \, \mu m \))
Cell type information
- Shape geometry at rest
- Volumes
- Materials

Environment information
- External & internal shapes

Cell population information
- Rates of each cellular phases (G0, G1 ...)
- Number of cell

CPOP
C++
Open source & free software

Force simulation
User interaction

G4World
GDML
Geant4

Input data
Output data
In-vitro spheroid culture simulate cell behavior using forces to generate a realistic cellular organization.

Generate cells with properties obtained by in-silico experimentations (life cycle, volumes...)

Add NP positionning (on membrane, nucleus...)

Export meshes and materials to Geant4 (G4World, GDML*)

* XML format based

The Cell POPulation modeler
**Spheroid & cell characterization**

- **Cell shape *in situ*** (confocal microscopy or SPIM)
  - Nuclear shape and dimension (DAPI)
  - Cell shape and dimension by cytoplasmic membrane staining (lipophilic fluorochrome)
  - Hypoxia evaluation *in situ* (Anh. Carb. IX activity)

- **Growth characterization**
  - Spheroid growth kinetic (Ø or volume)
  - Doubling time of cells (DNA or protein content)
  - Cell cycle analysis after dissociation (FCM)

*Data from in-vitro experimentations.*

*IMARIS data analysis*
MULTI-SCALE GEANT4 MONTE CARLO SIMULATION
GEANT4 DNA

Geometry
Realistic cellular models, DNA models

Physics models:
Classical and quantic for water and DNA

Experimental validation
Evaluate the damages occurred to cell and DNA

Water radiolysis
HO\textsuperscript{-}, H\textsuperscript{\cdot}, H\textsubscript{2}O\textsubscript{2}\textsuperscript{-}, R-O-O-H\textsuperscript{\cdot}

Karamitros et al. 2011
Prog. Nucl. Sci. Tec. 2

Expected in Geant4 version beta
June 2014

Incerti et al. Med. Phys. 2010
Spheroid irradiation

• X-RAD 320 system @ PAVIRMA
  – Voltage: 5 to 320 kV
  – Intensity: 0.1 to 45 mA
  – Circular field: radius 14 to 50 cm
  – Dose rate up to 13 Gy / min (5kGy max)

• Calibration
  – 250 kV RX beam

• Monte Carlo modeling
  – GATE/Geant4: Livermore models
  – 4x10⁹ primaries (<1% uncertainty)
  – Metrics: production of secondaries

Energy of generated primaries (keV)

Energy of charged secondaries (keV)
Water, 1 mm depth
Energy deposition per cell

- **PAVIRMA beam characteristics**
  - $E_{\text{max}}$ 250 keV: photoelectric effect
  - 2mm Al filter
  - Field size: 14.15 cm
  - SSD = 50 cm
  - Beam quality constant over 1 mm

- **Energy deposition**
  - Geant4 10.0.p01, Livermore models
  - Energy deposition in 10 µm diameter cell
  - Need to add NPposition
Gadolinium NP simulation

- **AGuIX AC13 nanoparticles**
  - 4Si-DTPA
    - ~3.5 nm diam. at pH = 7.4
    - 8.5 kDa
    - 21 TEOS, 18 APTES, 10 DTPA, 10 Gd, fraction in mass of Gd = 12%
  - Optimal energy to get differential effect ~50 keV

- **Monte Carlo modeling**
  - Geant4.10.00.p01, Livermore + fluo + auger
  - Metrics for preliminary study:
    - Production of charged secondaries
    - Number of photoelectric events in the nanoparticle
Secondaries produced by NP

Photoelectric event per primary

<table>
<thead>
<tr>
<th>Primary</th>
<th>NP Si-DTPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma 52 keV</td>
<td>1.8x10^{-5}</td>
</tr>
<tr>
<td>PAVIRMA</td>
<td>1.3x10^{-6}</td>
</tr>
</tbody>
</table>

Energy of charged secondaries (keV)
DNA DAMAGE PREDICTION USING GEANT4-DNA
DNA geometry and direct damages

- Atomic representation of DNA based on PDB

At the moment

- Direct damages in a dinucleosome using X-ray irradiator
  - SSB = 85 ± 1 /Gy/Gbp
  - DSB = 3 ± 1 /Gy/Gbp

To do

- Expand the nucleosome geometry
- Include indirect damages

- Geant4 advanced example to be provided in the next release (December 2014)
PERSPECTIVES
Perspectives

• Production of biological data for radioresistant cells
  – Using or not Gd nanoparticles
  – Morphology of spheroids, survival rates, production of foci

• Develop tools / methodology for a multi-scale modeling
  – Simulation of realistic Gd distribution
  – Calculation of direct DNA damages
  – Tools provided to the scientific community via the Geant4-DNA collaboration

• Correlation of experiments with simulations