Radiation Dose is More than A Number
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Cell Culture Studies

presented by

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What is being irradiated In Vitro

In Vitro cell culture - fortunately is no longer cells growing ‘on glass’ but ‘on plastic’.

Cells are either attached to a plastic surface (typically about 1 mm thick) or suspended in an aqueous medium.

For the attached method, cells are located as a very thin layer (~5 to 10 um in thickness) on the surface of a polystyrene sheet and are covered by an aqueous medium of thickness 1 to 5 mm.

The cell containers take various forms: dishes, flasks, multi-well plates, etc.
<table>
<thead>
<tr>
<th>type of flask, dish or plate</th>
<th>diameter (mm)</th>
<th>area (cm$^2$)</th>
<th>volume (ml)</th>
<th>thickness (mm) of medium above</th>
</tr>
</thead>
<tbody>
<tr>
<td>T175</td>
<td>175.0</td>
<td>175.0</td>
<td>35.0</td>
<td>2.0</td>
</tr>
<tr>
<td>T150</td>
<td>150.0</td>
<td>150.0</td>
<td>30.0</td>
<td>2.0</td>
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<tr>
<td>T75</td>
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<td>75.0</td>
<td>15.0</td>
<td>2.0</td>
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<tr>
<td>T25</td>
<td>25.0</td>
<td>25.0</td>
<td>10.0</td>
<td>4.0</td>
</tr>
<tr>
<td>T12.5</td>
<td>12.5</td>
<td>12.5</td>
<td>5.0</td>
<td>4.0</td>
</tr>
<tr>
<td>150mm</td>
<td>150</td>
<td>176.6</td>
<td>25.0</td>
<td>1.4</td>
</tr>
<tr>
<td>100 mm</td>
<td>100</td>
<td>78.5</td>
<td>10.0</td>
<td>1.3</td>
</tr>
<tr>
<td>60 mm</td>
<td>60</td>
<td>28.3</td>
<td>5.0</td>
<td>1.8</td>
</tr>
<tr>
<td>25mm</td>
<td>25</td>
<td>4.9</td>
<td>2.0</td>
<td>4.1</td>
</tr>
<tr>
<td>6 well</td>
<td>35</td>
<td>9.6</td>
<td>2.0</td>
<td>2.1</td>
</tr>
<tr>
<td>12 well</td>
<td>22</td>
<td>3.8</td>
<td>1.0</td>
<td>2.6</td>
</tr>
<tr>
<td>96 well</td>
<td>6.5</td>
<td>0.3</td>
<td>0.2</td>
<td>6.0</td>
</tr>
</tbody>
</table>
Goals of In Vitro Irradiation

1) Reproducible Dose
   a) Currently available
   b) Problem when changing radiation instruments

2) Uniform Dose
   a) Currently available - but user must be careful

3) Accurate Dose
   a) Currently available - but user must be very careful

4) Translate Dose conditions to In Vivo situation
   a) Available with careful help from physicist
Typical path to In Vitro Irradiation

Vendor Rep Promises

a) ease of use
b) stability
c) accurate dose
Typical path to In Vitro Irradiation

Vendor Provides

a) Radiation Device
b) Dose Rate in Air under very specific condition
c) Usable area for uniform dose
d) Means of verifying stability of dose rate
Everything is fine until:
Evil physicist shows up talking about

a) electron equilibrium
b) depth dose
c) Build-up
d) Mass attenuation coefficients
e) heal effect, kerma, ---- etc---------
What is role of Physicist?

a) Confirm that basic parameters provided by manufacturer are true;
   Reference dose to AAPM TG61
b) Define and analyze Standard experimental conditions that meet researchers “goals”
c) Analyze Non-Standard experimental conditions
d) Communicate
What are Physicists’ Tools

a) Ionization Chamber
   (absolute reference)
   (low resolution measurement)

b) GAFChromic film
   (cut to desired size; 2D dosimetry)
   (thickness and material simulates cells)
   (complicated to use - measuring dose requires expertise)

c) MonteCarlo dose modeling
   (treatment planning systems in development for this application)
Setup for AAPM TG61 measurement
What is Important for In Vitro Dosimetry?

a) Build-up?
b) Attenuation?
c) Depth Dose?
d) Backscatter?
e) Uniformity?
f) Photon energy
a) In dishes or flasks:
depth of aqueous medium above cells typically varies from 1 to 5 mm
GAF CHROMIC EBT2

Quality dosimetry plus:
- Self developing
- No processing required
- Energy independent
- Water resistant
- Now - More stability in room light
- Built-in uniformity enhancement

LOT# A090310-1B

25 SHEETS, EACH 8" x 10"
Dish on
Solid water
Depth Dose of 220 kvp X-rays in Solid H2O

- on 10 cm solid water
- Poly. (on 10 cm solid water)
- Poly. ()

Relative Depth Dose

Depth of Solid H2O above EBT2 film on 100 mm dish (mm)
100 kVp X-rays - on 10 cm solid water

Relative Dose

Depth of Solid H2O (mm) above EBT2 Film

- on 10 cm solid water
- Poly. (on 10 cm solid water)
Backscatter Material

The dose modifying effects of the material on which flasks or dishes are placed during irradiation is not sufficiently appreciated by many researchers. This backscatter material may vary from Stainless steel, to foam, to dense plastics, and who knows what else.
Dish on Foam (in air)
Chamber on Stainless steel
Role of Back-Scatter Material on Dose-Rate to 100 mm Culture Dish
220 kVp x-rays (HVL 9.5 mm Al)

- Ion chamber on surface
- EBT2 film under 2mm sH2O

<table>
<thead>
<tr>
<th>Back-Scatter Material</th>
<th>Relative Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG61</td>
<td>1.000</td>
</tr>
<tr>
<td>Surface of solid water</td>
<td>1.000</td>
</tr>
<tr>
<td>Soft foam (air)</td>
<td>0.800</td>
</tr>
<tr>
<td>Stainless steel</td>
<td>0.700</td>
</tr>
</tbody>
</table>
Role of Back-Scatter Material on Dose-Rate to 100 mm Culture Dish
100 kVp xrays (HVL - 5 mm Al)

Relative Dose

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ion chamber on surface

EBT2 film under 2mm sH2O
Depth Dose of 220 kvp X-rays in Solid H2O

- Relative Depth Dose vs. Depth of Solid H2O above EBT2 film on 100 mm dish (mm)
- Depth Dose of 220 kvp X-rays in Solid H2O on 10 cm solid water foam (air) stainless steel Poly. (on 10 cm solid water) Poly. (foam (air)) Poly. (stainless steel) Poly. ()
Depth Dose of 100 kvp X-rays in Solid H2O

Relative Depth Dose

Depth of Solid H2O above EBT2 film on 100 mm dish (mm)
The above was for simplest geometry. What about more complex

a) What is dose at edge of dish?

b) How does complexity of multiwell plates affect dose?

c) Should dishes be stacked?
96 well plate 220 kVp
EBT2 film on bottom of dish

0.2 ml of H2O on left side wells ---- right side empty.
220 kVp X-ray - EBT2 film on bottom of 96 well plate

Dose               351 cGy       362 cGy       320 cGy
Relative Dose      0.97          1.0           0.89
96 well plate  100 kVp
EBT2 film on bottom of dish

0.2 ml of H2O on left side wells ---- right side empty.
100 kVp X-ray - EBT2 film on bottom of 96 well plate

<table>
<thead>
<tr>
<th></th>
<th>water well</th>
<th>Empty well</th>
<th>wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>389 cGy</td>
<td>423 cGy</td>
<td>367 cGy</td>
</tr>
<tr>
<td>Relative Dose</td>
<td>0.92</td>
<td>1.0</td>
<td>0.86</td>
</tr>
</tbody>
</table>
What is important

a) Back scatter material $> 20$

b) Medium depth $< 5$

c) Complex object (96 well plate)

d) Beam quality
What is important

Determine needs of researcher

a) How accurate does dose need to be? Some experiments may need 2% whereas others need 20%

b) Will cold spots cause problems?
What is the role of Biologists in dosimetry and QA

a) Budget requires that biologists do much of their own routine QA
b) Biologists should not do complex physics tasks such as GAFChromatic film dosimetry
c) Biologists should be aware of basics and know what “not” to do
What should be done to improve the current situation?

a) Do nothing – no gain
b) Do everything for free – will not happen
c) Physicists aid when asked and available – current situation in large academic centers (limited impact over all)
d) Require training of researchers
e) Provide funds to researchers for physics QA support
Other things physicists can do?

a) Define class solutions
b) Recommend “do’s” and “do not’s”
c) Offer special physics services for custom problem solving
How to replace Cs-137 Irradiators?

a) Single or Duel Tube X-ray?
b) kVp Energy?
Gammacell 40 --- relative dose for single or 4 in stack dishes

- Single dish: 1.00
- Bottom of stack: 0.98
- #2: 0.93
- #3: 0.92
- Top of stack: 0.92

**Note:** The chart shows the relative dose for single or 4 dishes in a stack configuration.