In vitro study of dendronized nanoparticles designed for theranostics in breast cancer context

Introduction
The aim of the Theraget project is to develop targeted multifunctional nanoplatforms that allow diagnosis, therapy (theranostic) and follow-up diagnosis in breast and ovarian cancer context.

Theranostic:
- Diagnostic with MRI (iron oxide nanoparticles IONPs)
- Therapy using magnetic hyperthermia, a local elevation of temperature (alternative magnetic field)[4]

In vitro experiments must be done first, such as cytotoxicity tests and evaluation of IONPs internalization.

Cytotoxicity
NPs cytotoxicity evaluated by neutral red assay (2 hours of exposure)

<table>
<thead>
<tr>
<th>Iron concentration (µg/mL)</th>
<th>NPs@DD (N = 2)</th>
<th>NPs@DD-Ligands (N = 7)</th>
<th>NPs@DD_cRGD_all (N = 4)</th>
<th>NPs@DD_P22_all (N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>100</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

IONPs internalization (pg / cells)

Internalization study
Iron internalized by MDA-MB-231 cells after 24h exposure of 50 µg/mL nanoparticles

<table>
<thead>
<tr>
<th>Nanoparticles type</th>
<th>Iron internalized (pg / cells)</th>
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<tbody>
<tr>
<td>NPs@DD (N = 1)</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>NPs@DD_P22 (N = 3)</td>
<td>1.72 ± 0.08 pg_{iron} / cell</td>
</tr>
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After 24 hours exposure to 50 µg/mL iron, MDA-MB-231 cells have internalized:
- IONPs@DD_P22 : 1.72 ± 0.08 pg_{iron} / cell
- IONPs@DD : 0.34 ± 0.14 pg_{iron} / cell

Material & methods
IONPs are
- Synthesis: thermal decomposition coated with dendrons[2]
- Conjugated to targeting ligands: cRGD and peptide 22 (which are recognized by integrin αvβ3 and EGFR)

Integrins αvβ3 ➔ overexpressed in tumoral environment for neovascularization[1,4].

Peptide 22 ➔ promising EGFR specific triple negative breast cancer cell binding peptide (5).

Neutral red: viable cells internalize the neutral red dye into lysosomes and keep it even after washing.

Internalization: measured using iron quantification in biological matrix method[6]

Discussion
Considering the number of replicates, it seems that:
- Peptide 22 is a good candidate to increase IONPs internalization into MDA-MB-231. However, this value could be greater as compared to literature.
- IONPs synthesis will be modified in order to increase the number of targeting ligands on the NPs surface.
- Our IONPs are not toxic below 150 µg/mL, after 2 hours of incubation.
- For next steps, we won’t use more concentrated solutions.
- More measurements must be done at exposure times up to 24h.

References