SERS Intracellular Monitoring of Galunisertib Release from Porous Diatomite Nanoparticles in Colorectal Cancer Cells.

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Outline

☞ Introduction & Motivation: Hybrid Inorganic Nanoparticles for Colorectal Cancer

☞ Fabrication and Characterization of the Galunisertib Delivery System

☞ Monitoring of Galunisertib Release via SERS and HPLC Techniques

☞ Reversion of Metastatic Phenotype Induced by the Nanosystem in Colorectal Cancer Cell Line

☞ Conclusions and Future Perspectives
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Conclusions and Future Perspectives
Inorganic Nanoparticles in Medicine

Inorganic Nanoparticles (NPs)

- Silicon
- Silver
- Gold
- Silica

Diagnosis  Imaging  Drug delivery  Targeted Therapy

- Excellent biocompatibility of silica
- Non-toxicity
- High-Porous surface area
- Thermal stability
- Chemical inertness
- Tailorable surface chemistry
- Low-cost production of NPs
Diatomite Nanoparticles For Colorectal Cancer

Colorectal Cancer Incidence

10.6%

Upregulation
Metastatic genes

Cytoplasm

TβRII

TβRI

Smad2/3

Smad2/3

Nucleus

Nanosystem

CRC cell

Motivation

Galunisertib LY 2157299 + Diatomite Nanoparticles DNP

Lowering Toxicity
Improving Efficacy
Targeted Therapy

SERS monitoring


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Nanosystem Fabrication and Characterization

First step of functionalization

Step 1:

DNPs + HAuCl₄ solution → DNP-AuNPs

Nanosystem Characterization

TEM Characterization

<table>
<thead>
<tr>
<th></th>
<th>Size (nm) (DS)</th>
<th>Z-Potential (mV) (DS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNPs</td>
<td>400 (50)</td>
<td>+20 (5)</td>
</tr>
<tr>
<td>DNP-AuNPs</td>
<td>400 (50)</td>
<td>-15 (10)</td>
</tr>
</tbody>
</table>

The mean radius of the AuNPs grown on the DNP was 16 (5) nm. The overall complex had a size of 400 (50) nm and a negative surface charge, due to the pegylated AuNPs.
Fabrication and Characterization of the Nanosystem

Second step of functionalization

Step 2:

DNP-AuNPs-LY + Gelatin solution → EDC/NHS → DNP-AuNPs-LY@Gel

Nanosystem Characterization

Dynamic Light Scattering (DLS) Characterization

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<tr>
<td>DNP-AuNPs</td>
<td>400 (50)</td>
<td>-15 (10)</td>
</tr>
<tr>
<td>DNP-AuNPs-LY@Gel</td>
<td>450 (50)</td>
<td>-7 (8)</td>
</tr>
</tbody>
</table>

Optical Characterization

![Optical Characterization](image)

TEM Image

![TEM Image](image)

FTIR analysis

![FTIR analysis](image)
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☞ Analysis of the Nanosystem Drug Release Profile via SERS and HPLC Techniques

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**In vitro Drug Release Studies by HPLC Technique**

To highlight the advantages of the gelatin capping, the release behavior of both the DNP-AuNPs-LY@Gel complex and DNP-AuNPs-LY (without the gelatin layer) was studied by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC).

**Drug release behavior of DNP-AuNPs-LY@Gel**

**Drug release behavior of DNP-AuNPs-LY**

Nanosystem Loading Capacity: 20 µg of LY/mg of DNPs
Intracellular Galunisertib Release Monitoring via SERS analysis

The enhancement factor of the LY Raman signal provided by the DNP-AuNPs-LY@Gel complex was studied by SERS before investigating the release profile of the developed hybrid nanosystem in colorectal cancer (CRC) cells.

The most intense SERS vibration was found at 1360 cm⁻¹ and was used for monitoring the LY intracellular release from the developed platform.

An efficient SERS intracellular tracing of LY was performed up to 48 hours in living CRC and quantified to provide a LY sensing resolution down to $7.5 \times 10^{-18}$ g.
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Nanosystem-Induced Reversion of CRC Metastatic Phenotype

Modulation of Metastatic Genes in the LS-174T Cell line

Internalization of Alexa-488-labeled nanosystem and Epithelial Transformation of CRC cells

*S. Managò et al. Manuscript Peer Reviewed by Small, 2021

*Nanosystem concentration was 50 µg/mL in all the studies. According to the HPLC analysis, 50 µM/mL of DNP-AuNPs-LY@Gel contains 2.5 µM of LY
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Conclusions

Achievements

✓ Development of a pH-responsive hybrid Galunisertib delivery nanosystem with a size of 450 (50) nm and a drug loading capacity of 20 μg·mg⁻¹

✓ Real-time monitoring of Galunisertib release in living cells thanks to the high-sensitivity of the hybrid DNP-AuNPs-LY@Gel complex.

✓ Enhancement of the therapeutic effect of and reversion from metastatic to epithelial phenotype in CRC cells after 48 hours of treatment with DNP-AuNPs-LY@Gel.

Work in Progress

• Functionalization of the complex DNP-AuNPs-LY@Gel with Anti-L1CAM antibodies to address Galunisertib release in malignant cells overexpressing L1-CAM.

• Assessing the therapeutic effects of the newly developed system on different cell lines (pancreatic and CRC cell lines)
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