Response of 3D tumors to Photodynamic Therapy evaluated with Raman microspectroscopy

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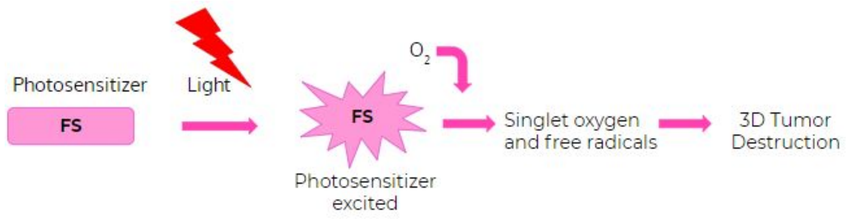
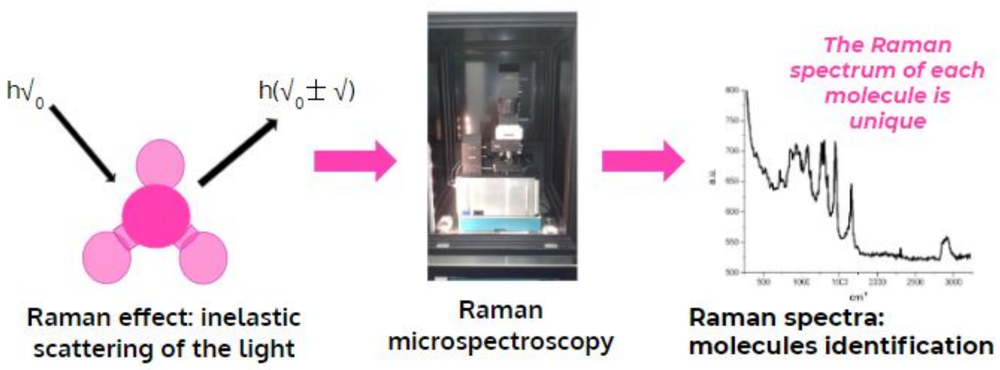
Introduction

Photodynamic Therapy is a technique used as an alternative for the treatment of various types of cancer in the clinic, including the breast cancer. It is important to understand the effects of PDT on solid tumors, since many studies are done on 2D culture and the 3D culture more closely resembles the natural tissue.

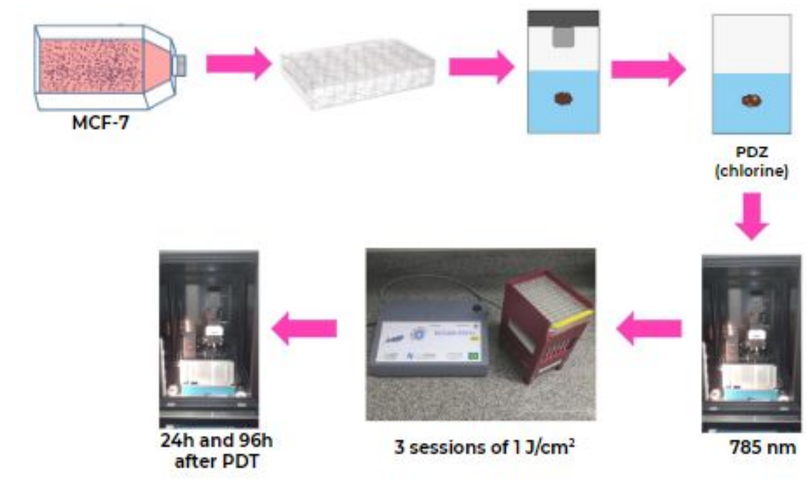
The purpose of this project is to evaluate the effects of Photodinamicy Terapy (PDT) in 3D cultures of tumor cells using the Raman microspectroscopy.



Raman microspectroscopy is one of the most suitable methods for discovery molecular mechanisms of the metabolic process and it is possible to study live cell samples without the use of markers.



Materials and Methods



Acknowledgements

CEPOF CENTRO DE PESQUISAS EM ÓPTICA E FOTÔNICA
bio photonics
FAPESP
CNPq
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IFSC

References

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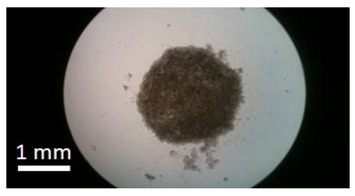
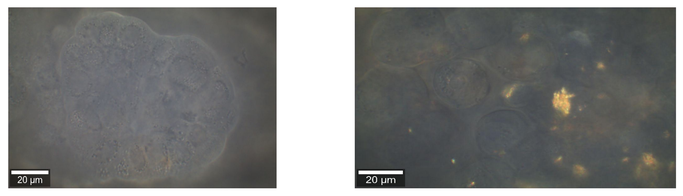
Results



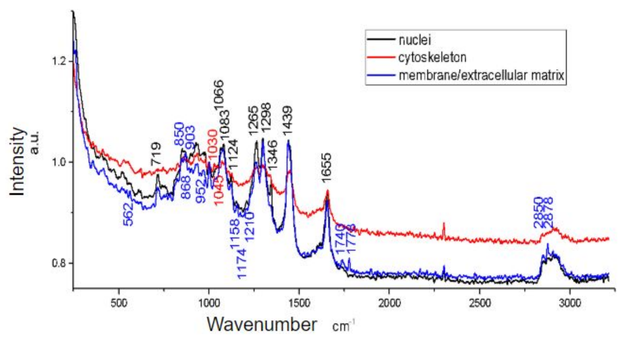
3D tumor after 4 days of growing.



Micrographs of 3D tumors before (left) and after (right) PDT.

Magnetic nanoparticles, used to form the tumor, work as a SERS nanoparticles in Raman microspectroscopy of spheroids and help to enhance the Raman signal from the cells.

Before PDT



The nuclei spectrum: nucleic acids (719, 1124, 1265, 1346, 1439 and 1655 cm^{-1}) [1]

The cytoskeleton spectrum: the bands and shape of the cytoskeleton spectrum in the study of Klein and cols [2]

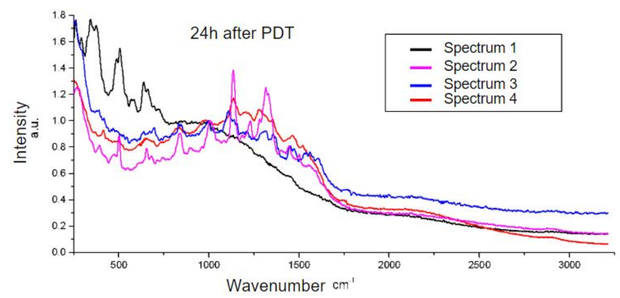
The membrane/ ECM spectrum:
glycine (562 and 1158 cm^{-1}) [1]
proline (850, 903, 952 and 1174 cm^{-1}) [1] ➡ Extracellular matrix
fibronectin (1740 cm^{-1}) [3]

lipids (2850 e 2878 cm^{-1}) [1] ➡ Membrane

After PDT

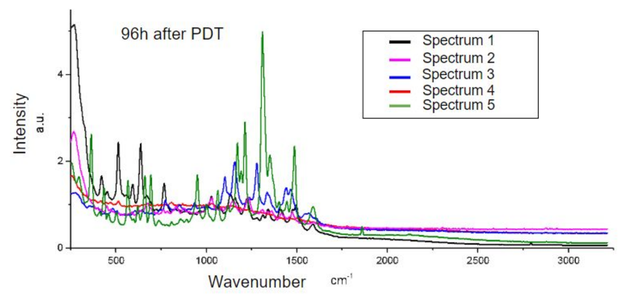
24h after PDT:
decrease in spectra:
- Lipids (2850-2880 cm^{-1}) [4], nucleic acids (1265, 1439 and 1655 cm^{-1}) [5]

increase in spectra:
- S-S bond of fibronectin (502-506 cm^{-1}) [4], [6]



Disaggregation of ECM and cellular membrane, exposing them to the environment.

96 hours after the PDT:
Increase of peaks associated with aromatic amino acids:
- Phenylalanine (363, 951, 1156 and 1351 cm^{-1}) [1]
- Tyrosine (431 and 1214 cm^{-1}) [1]
- Tryptophan (594, 768, 1102, 1278, 1310 and 1486 cm^{-1}) [1]



Conclusion

With Raman microspectroscopy it is possible to distinguish cellular components and monitor changes in cells after Photodynamic Therapy in 3D culture models. In our study we show that both tumor cells and extracellular matrix are damaged with PDT.