3D CULTURE MODELS-NEW SCREENING PLATFORM FOR NATURAL PRODUCTS

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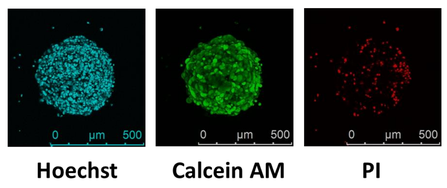
Cancer continues being one of the main causes of death worldwide, whose incidence is estimated to increase in the coming years. Given this situation, the development of new therapeutic strategies is of great importance, among which **Natural Products (NPs)** derived from the secondary metabolism of bacteria and fungi are being widely explored. **Fundación MEDINA** is an independent non-profit Research Organization created to discover new compounds and innovative therapies for unmet medical needs. It is leader in **Drug Discovery** from Microbial Natural Product Libraries being exclusive owner of one of the world´s largest **Microbial Collections** (190.000 microbial strains), and **Natural Products Libraries** (over 200.000 extracts & fractions)[1,2]. Here, we show of the characterization and establishment of **3D culture models** (spheroids) in tumor cell lines in a reproducible way that is compatible with high-content bioimaging systems. Finally, a pilot **High-Throughput Screening (HTS)** assay was performed on a library of 320 microbial extracts from the **MEDINA collection**, which allowed early detection of extracts with antitumor activity.

INTRODUCTION

MEDINA's main objective is to discover molecules with attractive biological and chemical properties as well as other therapeutic approaches suitable to industrial exploitation.

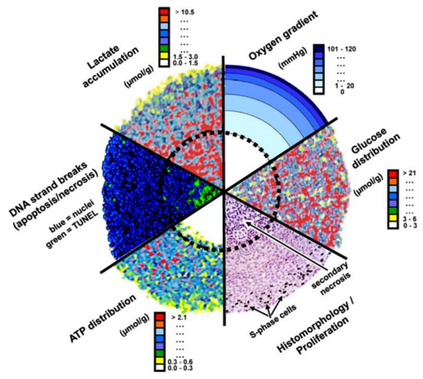
Cell-based assays have become a key element in drug discovery and therapy test programs[3,4].

3D cultures (spheroids) are introduced in the antitumor drug screening process at MEDINA as they mimic *in vivo* physiology incomparably better than the standard 2D culturing.



Images of a single spheroid of MCF-7 cell line stained with Hoechst (blue), Calcein AM (green) and propidium iodide (red) by confocal microscopy. Ten images of a single spheroid stained with Hoechst by confocal microscopy (LEICA SPE plus HCS A module, 10x) in the range Z = 0 – 150 µm, used to merged using ImageJ software

Analytical images of spheroid median sections



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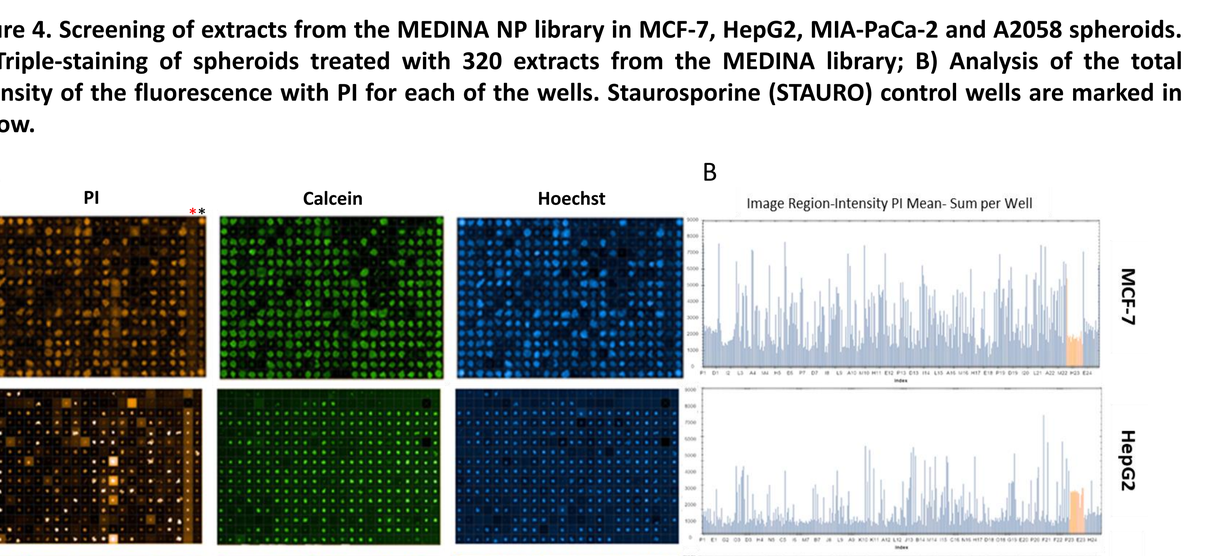
MATERIALS AND METHODS

New systems such as the Echo® 550 Acoustic Liquid Handler (Beckman Coulter) and Operetta CLS High Content Analysis System (PerkinElmer) were used in this screening.



In this work, spheroids have been established in the cancer cell lines **MCF-7**, **HepG2**, **MIAPaCa-2** and **A2058**. A screening of 320 microbial extracts from NPs from Fundación MEDINA, which had previously been shown to have activity in 2D cell models, was carried out.

RESULTS

✓ Optimization, Validation and Characterization of Spheroid Cultures

Figure 1. A) Staining of the spheroids and visualization of the inner and outer regions for fluorescence quantifications; B) Steps followed in the image analysis protocol

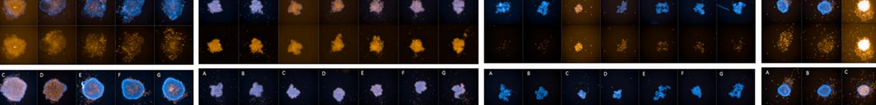


Figure 2. PI inner and outer spheroid signal with reference compounds

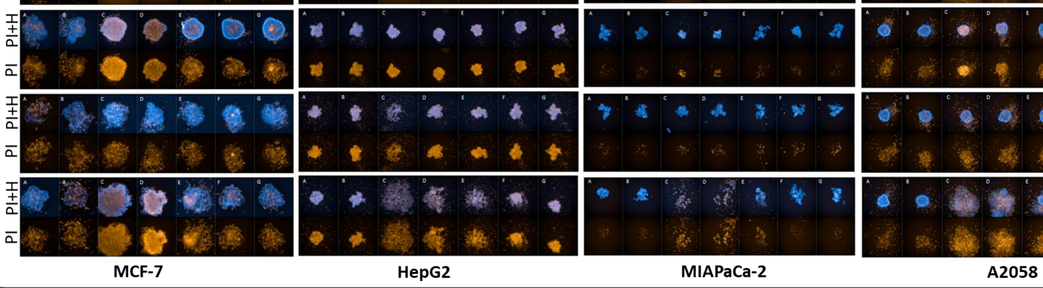


Figure 3. Staining method of spheroids with reference compounds

✓ High-Throughput Screening Pilot with extracts from the MEDINA Natural Product Library

Figure 4. Screening of extracts from the MEDINA NP library in MCF-7, HepG2, MIA-PaCa-2 and A2058 spheroids. A) Triple-staining of spheroids treated with 320 extracts from the MEDINA library; B) Analysis of the total intensity of the fluorescence with PI for each of the wells. Staurosporine (STAURO) control wells are marked in yellow.

Table 1. Comparison of the number of active extracts in the 2D and 3D culture models

	MCF-7		HepG2		MIAPaCa-2		A2058	
Culture models	2D	3D	2D	3D	2D	3D	2D	3D
Total active extracts	47	60	66	60	58	15	60	10

CONCLUSIONS AND FUTURE PERSPECTIVES

- In this work, it was possible to obtain reproducible spheroids in 384-well plates of four different cancer cell lines, allowing the pilot screening **of 320 extracts** from **MEDINA Natural Product library** derived from **actinomycetes and fungi.**
- In future research, the confirmation of the active extracts through cherrypicking studies will be continued. In addition, the compounds responsible for the activity will be identified and isolated through bio-guided fractionation and their functional validation performed.
- The **establishment of spheroid culture models** carried out in this project in the human cancer lines MCF-7, HepG2, MIAPaCa-2 and A2058 together with the **implementation of high-content bioimaging systems**, represents a **promising tool for the evaluation of new treatment strategies in a 3D cellular context capable of mimic characteristics of solid tumors**.

REFERENCES

[1] J Biomol Screen. 2016 Jul;21(6):567-78. [2] Exp. Cell Res. 2014; 323, 131–143. [3] Journal of Biotechnology. 2010; 148(1), 3–15. [4] Assay Drug Dev. Technol. 2015; 13, 402–414.

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