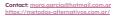
THP-1-Derived Macrophages Integrated into a 3D Hepatic Spheroid Model for Toxicity **Assessment**

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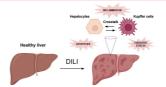




INTRODUCTION

Drug-induced liver injury (DILI) is a leading cause of drug withdrawal and a major concern in drug safety. Hepatocytes are the primary targets of toxicity, while Kupffer cells, the liver's resident macrophages, play a key role in modulating inflammatory responses.

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Traditional animal-based toxicity testing often lacks predictive power and involves ethical concerns. Therefore, developing human-relevant in vitro models that incorporate both parenchymal and immune components is essential to better predict hepatotoxicity and improve drug safety.

MATERIALS AND METHODS

Differentiation of THP-1 cells into macrophages a-CD86/FITC THP-1 monocytes seeding 冢 48 h treatment 24 h rest period Cell counting Cell staining Cell differentiation with PMA

Data analysis

HepG2 cells THP-1-derived macrophages Cell seeding Seeded in agar-coated 96-well plate Initial cell number 100 1000 3000 HepG2:THP-1 ratios 100:0 80:20 60:40 Spheroid formation 🔀 3 days Monitoring up to 14 days Morphological changes (diameter, sphericity, compactness)

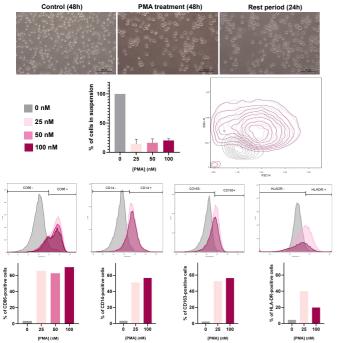
Hepatic spheroid formation

RESULTS

Differentiation of THP-1 cells into macrophages

Flow cytometry

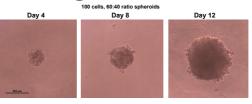
acquisition

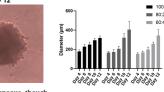


- Morphological shift from suspension to adherent phenotype after PMA treatment
- Decreased percentage of cells in suspension compared to control
- Flow cytometry (SSC vs FSC) revealed increased cell size, granularity, and higher population heterogeneity.
 Expression of four macrophage surface markers increased after PMA exposure.

Hepatic spheroid formation

Time





Viability assays (MTT, NRU)

- Spheroids seeded with 100 cells were more spherical and homogeneous, though requiring longer aggregation time.

 Spheroids seeded with 1000–3000 cells failed to acquire a well-defined
- Inclusion of macrophages improved cell aggregation and spheroid roundness.
- Spheroid diameter progressively increased over time, indicating growth and
- stability.
 Cell viability confirmed by MTT and NRU staining.

SIGNIFICANCE AND PERSPECTIVES

This work marks a step forward in establishing a 3D hepatic model, aimed at improving in vitro toxicity testing by better capturing human physiology. These advances open the path for the next steps in model refinement and application.



Co-culture

- Spheroid bioimaging for cell types distribution and viability
 - [Albumin] for protein
- [Urea] for amino acid
 metabolism
- CYP3A4 activity for xenobiotic
- RNAseq for transcripcional

