

# From a 3D-model of particle-induced granuloma-like structure to a simple macrophage bioassay predicting granulomagenic and fibrotic activity of particles

Created on: 23-03-2023 - Last modified on: 03-04-2023

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## Organisation

**Name of the organisation** Université Catholique de Louvain (UCL)

**Department** louvain center for toxicology and applied pharmacology (LTAP)

**Country** Belgium

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Basic Research, Regulatory use - Routine production
<b>Type of method</b>	In vitro - Ex vivo

## DESCRIPTION

### Method keywords

spheroids

agarose

ELISA

cell culture

confocal

## **Scientific area keywords**

lung toxicology

predictive toxicology

inhaled particles

lung diseases

## **Method description**

Macrophages orchestrate reactive particle segregation, compact aggregates of immune cells and non-immune cells and promote fibrosis-surrounding granulomas. We developed a simple 3D in-vitro model that mimics granuloma formation and categorizes granuloma-inducing inorganic particles. Macrophage cell line (MHS) pre-exposed for 24h to 10µg/mL of granuloma-inducing (Carbon nanotubes, CNT) or not (Carbon black,CB) particles are cocultured with fibroblasts and epithelial cells (respectively MLG and LA4 cell lines) on 0,3% agarose coated wells. Fluorescent dyes and confocal microscopy showed that these cells in presence of CNT but not CB were organized in layered compact cellular aggregates comparable to granulomas after 7 days. The supernatant collected at 24hours (but also at 78hours and 7days) contains significantly elevated levels of the pro-fibrotic mediator TIMP1 (metallopeptidase inhibitor 1) only in granuloma-inducing conditions (CNT). The levels of other pro-granulomagenic and fibrotic mediators (such as matrix metalloproteinase 1, MMP-1; Osteopontin, OPN or the chemokine CCL2) were not increased. Our data suggest that macrophages combined to structural cells respond to granuloma-inducing particles by releasing TIMP-1 and organizing in vitro granuloma-like spheroids. This model was further simplified, as MHS macrophages alone were sufficient for the specific release of TIMP-1 in response to granulomagenic particles. Quantification of macrophage-produced TIMP-1 is a novel and simple tool for predicting and assessing granuloma-inducing new material and airborne dust particles.

## **Lab equipment**

ELISA cell culture infrastructure (confocal) microscopy

## **Method status**

Still in development

Internally validated

## **PROS, CONS & FUTURE POTENTIAL**

### **Advantages**

- In vitro method - no animal method : cell lines - Simple

### **Challenges**

Coating a flat agarose layer is necessary for observing a basal uniform cellular layer

### **Modifications**

Refinement of the three cellular model to a unicellular model

### **Future & Other applications**

Exposition to a wide range of reactive materials

## **REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION**

### **Associated documents**

[CB \(not-granuloma-inducing\) - no spheroid.avi](#)

[vehicle - no spheroid.avi](#)

[CNT \(granuloma-inducing\) - granuloma-like spheroid.avi](#)

[From a 3D-model of particle-induced granuloma-like structure to a simple 2D-macrophage bioassay predicting granulomagenic and fibrotic activity of particles.pdf](#)

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