

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

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## **Contact person**

Léa Hiéronimus

## **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

| The Method relates to     | Human health                                        |
|---------------------------|-----------------------------------------------------|
| The Method is situated in | Basic Research, Regulatory use - Routine production |
| Type of method            | 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 granulomainducing 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 profibrotic 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

Profinate 3 by-model of particle-induced granting 1988 - 1986 structure to a simple 2D-









