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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Organisation

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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 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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