Effects of polyester microfibers released from a domestic dryer machine on human lung organoids and Xenopus laevis

Anna Winkler [a], Nadia Santo [b], Laura Madaschi [b], Alessandro Cherubini [c], Francesco Rusconi [c], Lorenzo Rosso [d], Paolo Tremolada [a], Lorenza Lazzari *[c], Renato Bacchetta **[a]

FONDAZIONE IRCCS CA' GRANDA
OSPEDALE MAGGIORE POLICLINICO
Sistema Sanitario Regione Lombardia

MOTIVATION

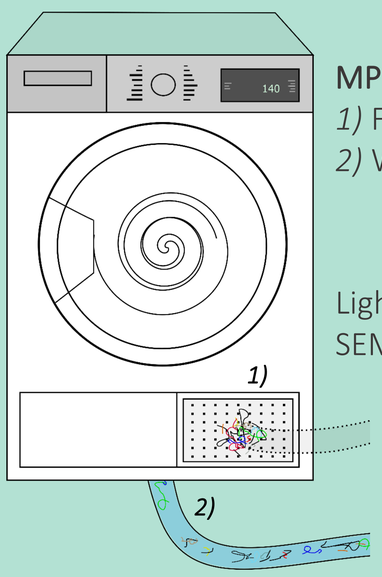
Microplastic fiber (MPF) emissions from dryer machines into air and waterways are poorly investigated. Thus, the objective was to characterise and quantify the release of polyester fibres from a domestic dryer machine and to analyse their effects on two test models representing potential targets of airborne and freshwater contamination; *in-vitro* human lung organoids and the *in-vivo* animal model *Xenopus laevis*. Since human organoids were not yet applied to evaluate the biological effect associated with MP/NP exposure, this study seeks to evaluate their applicability and suitability in microplastic exposure tests.

Human organoids

- Most advanced *in vitro* model
- Self-organizing 3D structure grown from stem cells
- Recapitulate essential aspects of the *in-vivo* organ structure and function while being more experimentally tractable than model organisms [1]

EXPERIMENTAL DESIGN

Drying of polyester clothes

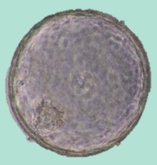


MPF collection from
1) Filter of exhaust air
2) Wastewater

↓

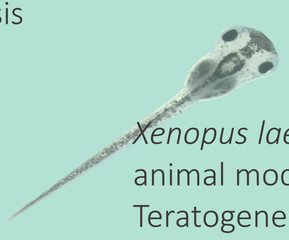
Light microscopy, SEM + DLS analysis

Exposure



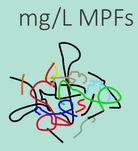
1, 10 and 50 mg/L MPFs

Human lung organoids; adult stem cell derived, obtained from healthy human lung tissues



Xenopus laevis; standardized animal model Frog Embryo Teratogenesis Assay-Xenopus, FETAX

Fixation



Confocal microscopy and SEM analysis

Optical microscopy and SEM analysis.
Analysis in progress.

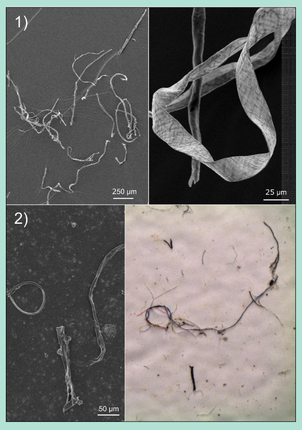
Immunofluorescence staining

To ensure the quality of organoids and to visualize the cell architecture, the cultures were stained with cytoskeleton markers and analysed with a confocal microscope.

Scanning Electron Microscopy (SEM) was also performed to observe the fine structure of the organoid surface.

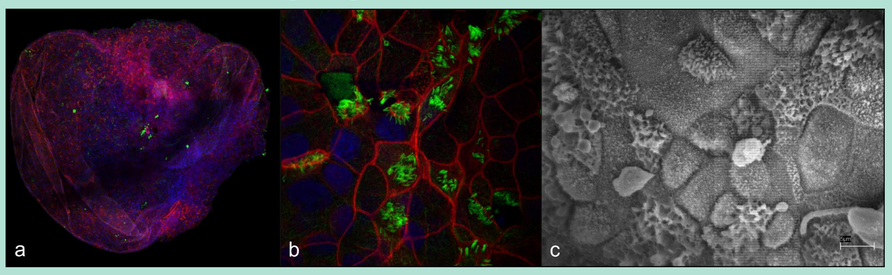
RESULTS *(preliminary)*

MPF release of a dryer machine

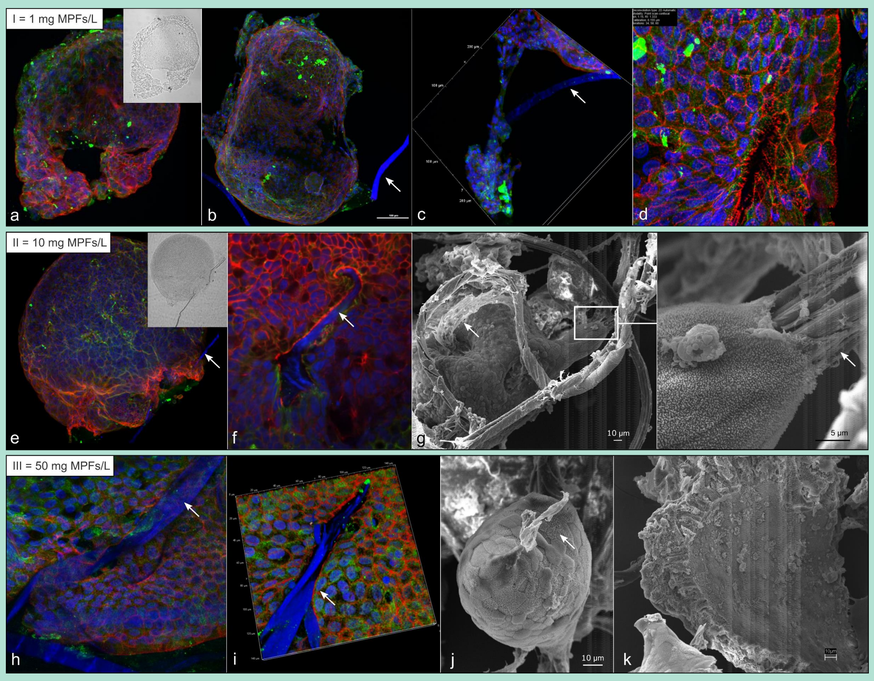


- The drying process emitted a total weight of 0.4 g/kg dry weight of MPFs into the exhaust air (*1*) and 1.24 mg/kg dry weight of MPFs into the wastewater (*2*).
- Mean Fibre length was 663 ± 333 µm.
- Interestingly, the shape of these MPFs differed at the transversal surface; exhibiting a varying profile from flat and twisted to tattered with a minimum height of 1-3 µm along the thinnest dimension.
- The hydrodynamic diameter ranged from 100 nm to 340 nm (referring to the diameter of a spherical particle of the same volume as the detected MPF).

Cultivation of lung organoids



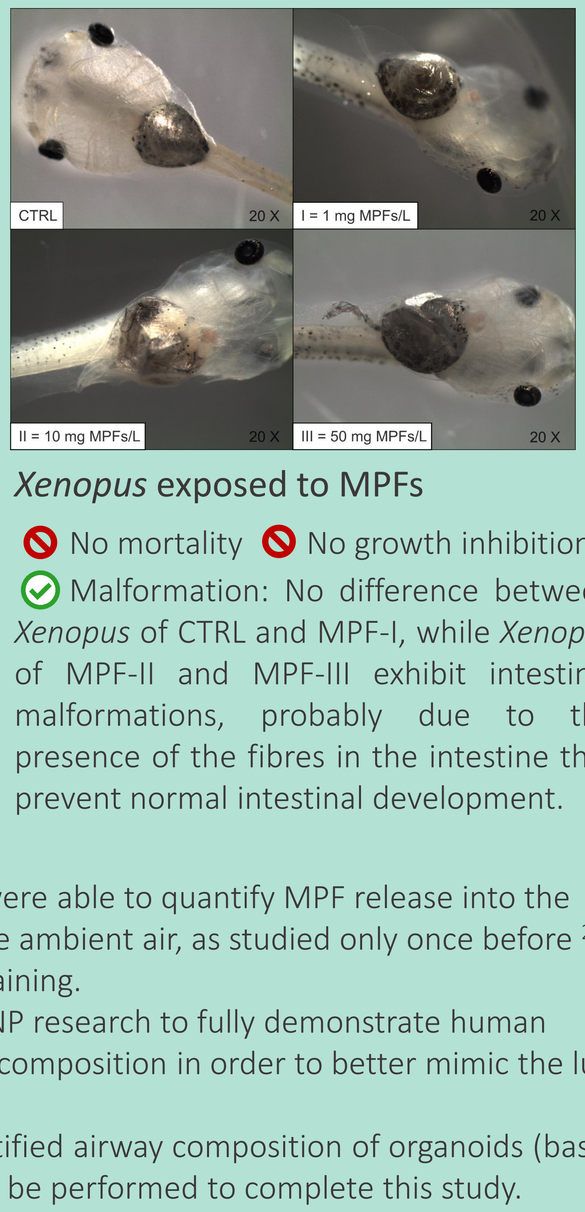
- (*a+b*) Confocal microscopy images of the 3D structure of immunolabelled lung organoid (control sample) and surface section of lung organoid showing markers for cilia (green, Alexa Fluor 488) and nucleus (blue, Hoechst 33342). Counterstained is the actin cytoskeleton (red, F-actin).
- Mature organoids are 400-600 µm in diameter.
- (c) SEM image of organoid surface demonstrating cell diversity.



Confocal microscope images and counter phase contrast (b/w) (left) and SEM images (lower right) of lung organoids after exposure to MPFs from a dryer machine at different concentrations. MPFs are indicated with a white arrow.

Organoids exposed to MPFs

- Lung organoids were affected by MPFs at all concentration steps.
- Organoids exposed to 1 mg/L MPFs exhibited deformation of their 3D structure, such as openings (*a*) and teared off cells (*c*).
- Interestingly, we observed an internalisation of the MPFs in organoids at the second and third exposure concentration (*e-g*). While some MPFs seem to be embedded in the surface of organoids (*h+i*), we can also observe a full internalisation of MPF sections (*g* and its magnification).
- Image *g* gives the impression that a MPF entered the organoid by pushing through the surface and exiting it again, while pulling along cell tissues creating a dip at the entrance and a bulge at the exit. This effect might be resulted by the dynamical processes of the MPFs being suspended in the solution and are free to move around.



Xenopus exposed to MPFs

No mortality No growth inhibition

Malformation: No difference between *Xenopus* of CTRL and MPF-I, while *Xenopus* of MPF-II and MPF-III exhibit intestinal malformations, probably due to the presence of the fibres in the intestine that prevent normal intestinal development.

CONCLUSION AND OUTLOOK

- The drying of synthetic clothes releases MPFs and contributes thus to the contamination of MP/NP in our environment. We were able to quantify MPF release into the wastewater. MPFs in the exhaust air were filtered by the dryer, therefore, the next step is quantifying the MPF release into the ambient air, as studied only once before [2].
- We cultivated human lung organoids and were able to display their cytoarchitectural organization by immunofluorescence staining.
- Organoids (3D model) have the potential to replace animal models and primary human tissues (cell lines, 2D models) in MP/NP research to fully demonstrate human physiological responses. However, the lung organoid model requires further advancement in the heterogeneity of its cellular composition in order to better mimic the lung tissue physiological functions. Other shortcomings remain; their accessibility and higher costs than conventional 2D models.
- To detect inflammatory cytokine in organoids (triggered eventually by tissue damage) and to further validate the pseudostratified airway composition of organoids (basal cells, functional multi-ciliated cells, mucus-producing secretory cells, and club cells), gene expression analysis by qRT-PCR will be performed to complete this study.
- Further tests on lung organoids will be performed with different shaped and sized synthetic polymers (i.e. nanospheres).

AFFILIATIONS

[a] Department of Environmental Science and Policy, University of Milan, Via Celoria, 26 - 20133 Milan, Italy.
Contact E-mail: *anna.winkler@unimi.it*
[b] Unitech NOLIMITS, Imaging facility, University of Milan, Via Golgi 19 - 20133 Milan, Italy
[c] Laboratory of Regenerative Medicine - Cell Factory, Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[d] Health Sciences Department, University of Milan and Thoracic surgery and transplantation Unit, Fondazione IRCCS Ca Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35, 20122 Milan, Italy
*co-last author for the human organoids, **co-last-author for microplastic and microscopy analyses

[1] Sachs et al. (2019) Long-term expanding human airway organoids for disease modeling, 38(4), EMBO J.
[2] O'Brien et al. (2020) Airborne emissions of microplastic fibres from domestic laundry dryers, Sci. Total Environ.