Electron microscopy studies of the bio-interactions of novel nanomaterials and *in vitro* models: the contribution of PMiB

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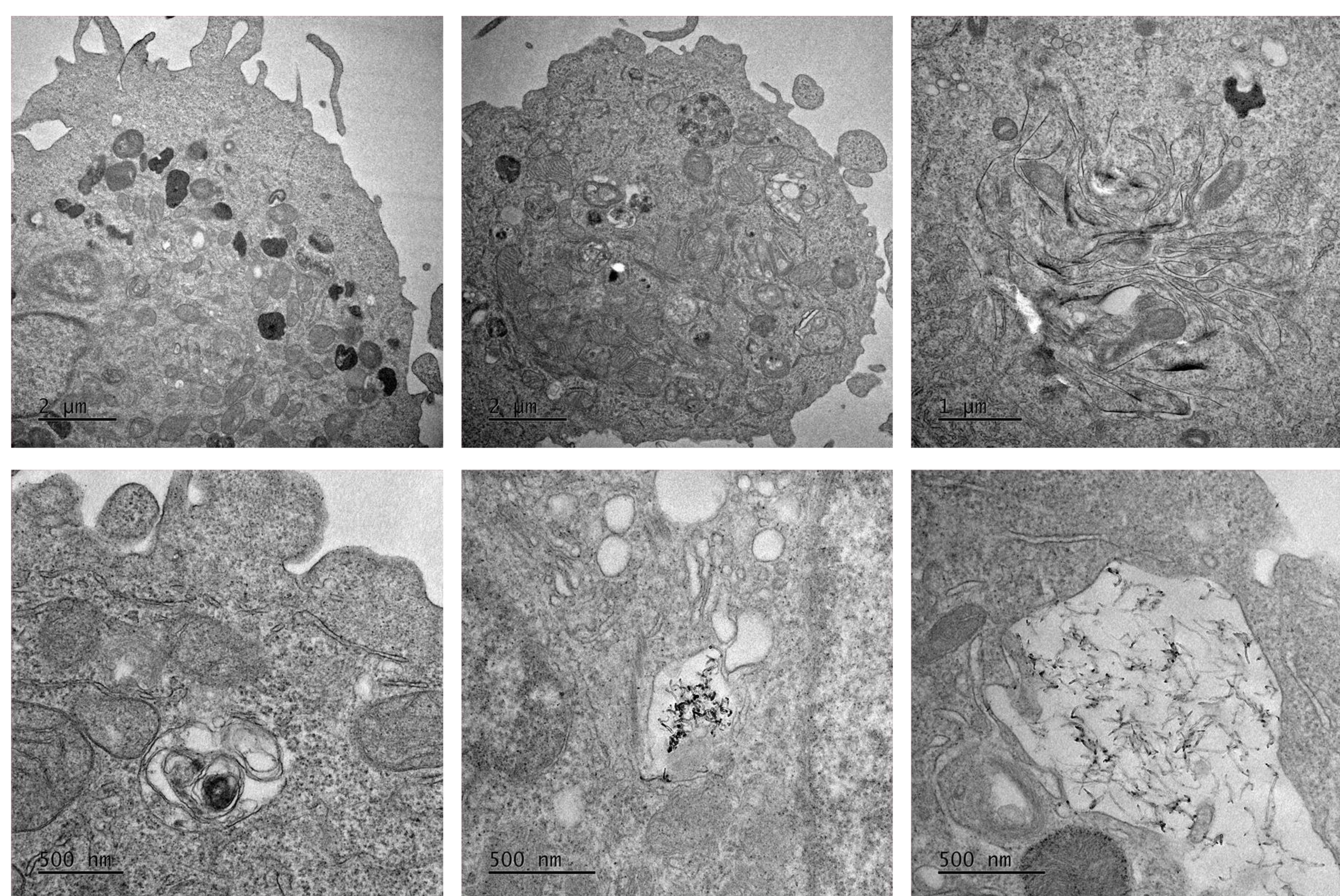
Introduction

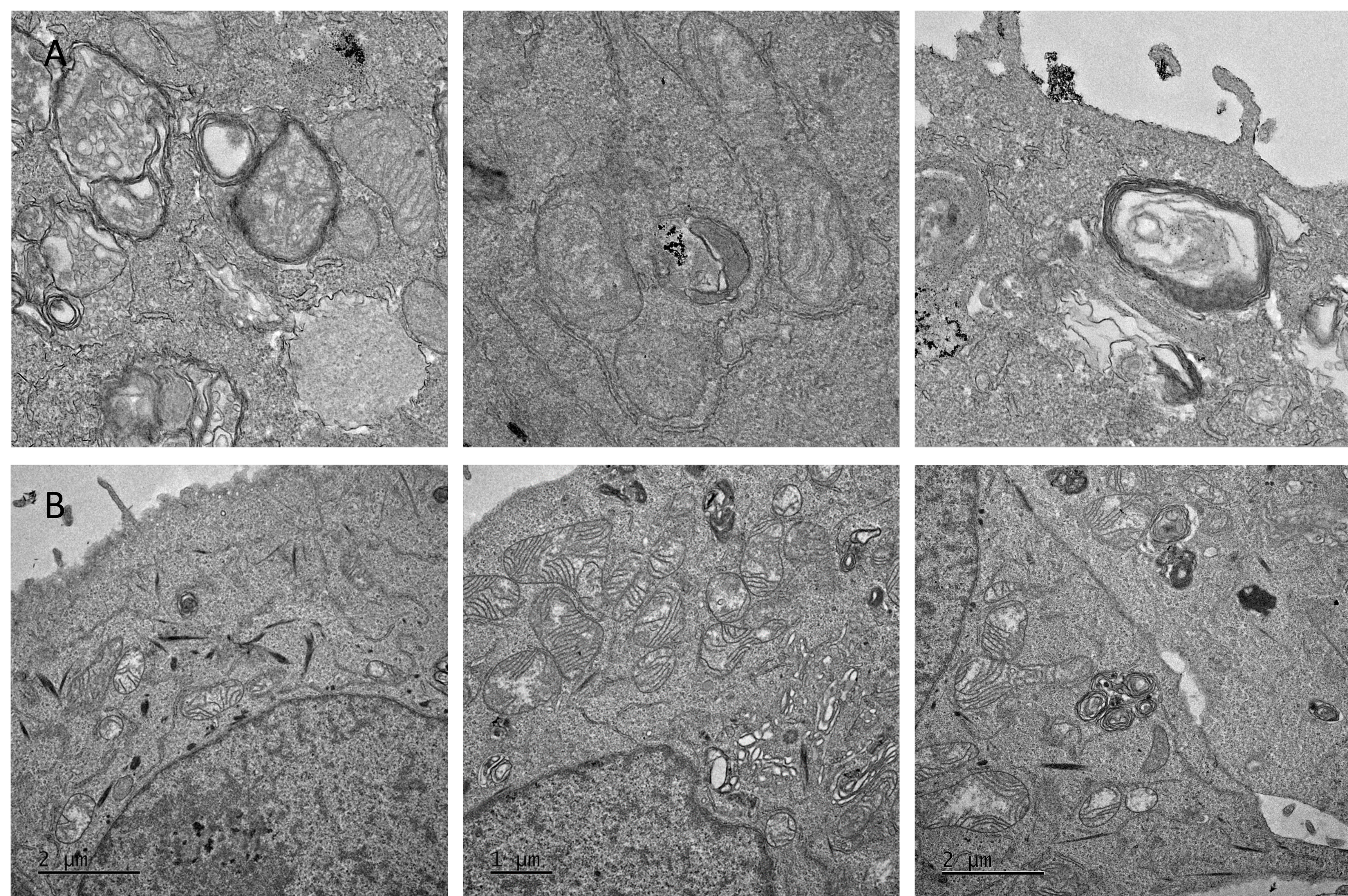
Electron Microscopy (EM) represents a powerful tool in biological studies to better understand how new nanomaterials (NMs) are able to interact with in vitro models and studying their effects. **Transmission Electron Microscopy** (TEM), with its high-resolution power (0.1 nm), allows to investigate bio-interactions between cells and NMs at the ultrastructure cellular level. Thanks to TEM analysis, it is possible to describe how NMs affect the cells and if they are up-taken by cells entering the cellular membrane. Once inside the cell, NMs can be accumulated at cytoplasmic or nuclear level, causing possible structural damages bringing also to organelle malfunctions or even DNA damages. **Scanning Electron Microscopy** (SEM), differently to TEM, does not allow an investigation at the cellular ultrastructure level, but it can give information regarding the cell surface. With SEM analysis, it is possible to investigate damages at the cell membrane level and if NMs interact with cellular surface structures (e.g. filopodia) giving a different and complementary perspective of the bio-interactions from TEM studies. Especially in nanotoxicological studies, knowing the structure and the composition of the NMs can be a relevant information. For these reason, Scanning Transmission Electron Microscopy (STEM) coupled with elemental analysis (e.g. Energy Dispersive x-rays Spectroscopy, EDS) can be used. It can univocally confirm the NM presence in the in vitro model both in TEM and SEM samples. In addition, EM can be useful even to first characterize alone the NM to be tested. The novel NMs can be described in term of dimension, morphology, crystalline structure (diffraction pattern) and chemical composition (elemental analysis, e.g. EDS). Here, we report some cases of EM studies involving different *in vitro* models and several innovative nanomaterials.

Transmission Electron Microscope (TEM), JEOL JEM 2100 Plus
Source: LaB_6 Filament
Spatial resolution: 0.24 nm
Accelerating voltage: 80 - 200 kV
Mode: TEM, STEM, Diffraction
Detectors: Bright Field (BF), High Angle Annular Dark Field (HAADF), Energy Dispersive System (EDS)

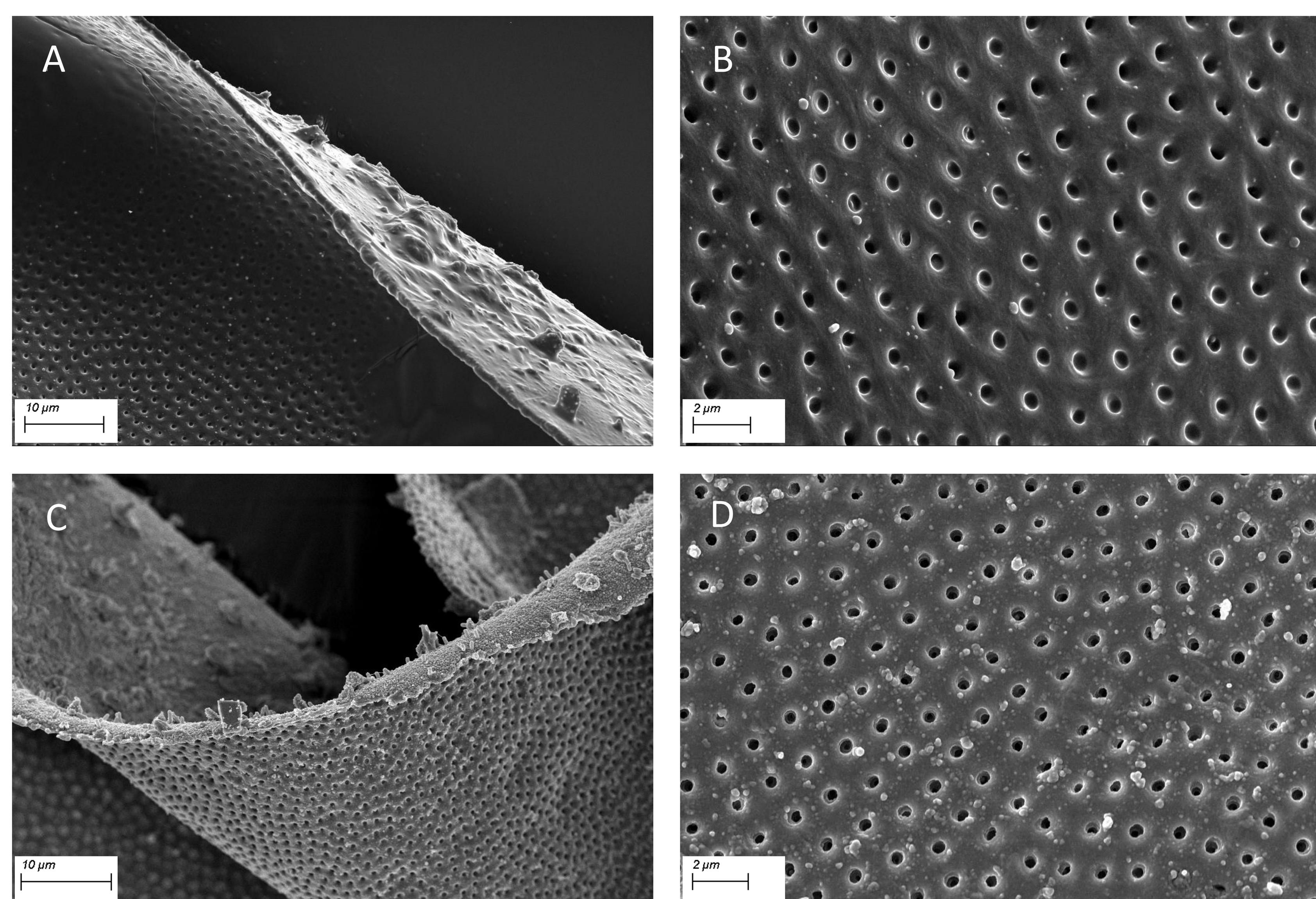
Scanning Electron Microscope (SEM), Zeiss Gemini 500
Source: Field Emission
Nominal resolution: 0.6 nm at 15 kV
Accelerating voltage: 0.5 - 30 kV
Mode: SEM, STEM
Detectors: Secondary Electrons (SE), Backscattering Electrons (BSE), Electron Backscattered (EBSD)



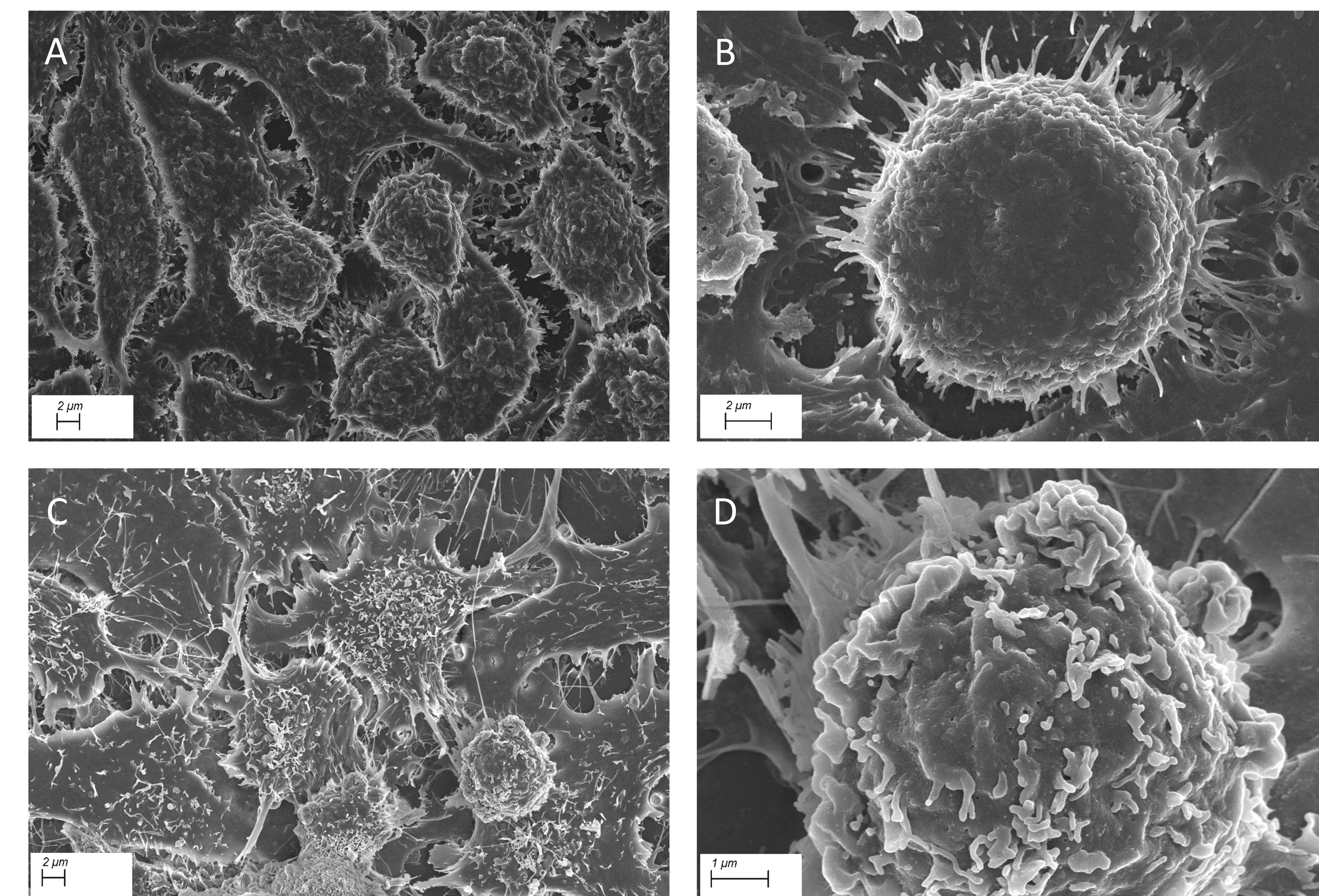
A549 cells (human lung adenocarcinoma cell line). Cell monolayers treated for 24 hours in submerged condition with carbon nanotubes (CNT)



A549 cells (human lung adenocarcinoma cell line). Cell monolayer treated for 24 hours in submerged condition with silver nanoparticles (AgNPs) coated with Hydroxyethyl Cellulose (HEC, A) or Polyvinylpyrrolidone (PVP, B)



Zebrafish chorion. A and B, chorion from embryos not treated, negative control group; C and D, chorion from embryos treated with silver nanoparticles (AgNPs) coated with Hydroxyethyl Cellulose (HEC)



A549 cells (human lung adenocarcinoma cell line) and THP-1 cells (human macrophage-like cells). A and B, control group; C and D, co-coltures treated for 24 hours with nebulized titania nanoparticles (TiO_2-N)

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PIATTAFORMA DI MICROSCOPIA DI MILANO-BICOCCA

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