Potential harmful effects of nanoplastics derived from teabags in an *in vitro* model of the intestinal barrier

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The increasing presence of micro- and nano-sized plastics in the environment, and in the food chain, is of growing concern. Plastics from consumer goods can break down into microplastics and nanoplastics (MNPLs) complicating their detection and quantification. One of the most popular goods used universally is the teabags, which is a new source of MNPLs since the traditional paper bags were substituted by plastic bags.

Among the different studies on the potentially hazardous effects of MNPLs, those related to the MNPLs released from teabags are practically inexistent. To cover this gap, we have designed a new method to first obtain, identify and characterize the plastic particles released from teabags, and then determine their harmful effects on human cells. Since humans are exposed to teabags-MNPLs via ingestion, we have used a well-stablished model of *in vitro* intestinal barrier as a target. The model consists in a triple coculture containing enterocytes (Caco-2 cells), goblet cells (HT-29 cells), and lymphocytes B (Raji-B cells).

To obtain the released MNPLs, we have used tea bags available from the supermarket. After removing the tea fraction, the teabags were washed 3 times with Milli-Q water and boiled in water at 95 ºC for 30 min. Finally, the sample was sterilized with 95 % ethanol and ultra-centrifuged to have enough volume and concentration to carry out the characterization and cell treatment procedures. The particles were fully characterized by TEM (Transmission Electron Microscopy), SEM (Scanning Electron Microscopy), SEM-EDX, Nano Z-sizer, and FTIR (Fourier Transform Infrared Spectroscopy).

The results from SEM-EDX and FTIR confirmed that the particles derived from teabags were mostly polylactic acid (PLA). Moreover, using the Nano Z-sizer we could detect a hydrodynamic size of approximately 100 nm. Finally, TEM and SEM images showed the spheric shape and confirmed the size from both PLA in suspension and in the teabag tissue.

Once the protocol for MNPLs has shown to be robust, and the overall characterization has been done, we aimed to test our own teabag-derived PLA-NPs on the *in vitro* model of the intestinal barrier Caco-2/HT29/Raji-B. Our hazard approach involves determining the toxicity, evaluating the effects on the barrier integrity and permeability, determining cell uptake and translocation, as well as potential induction of oxidative stress and genotoxicity.

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