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Studying the effects of nanoplastics derived from teabags in an in vitro model of the intestinal epithelium

G. Banaei^{1*}, A. Villacorta^{1,2}, A. Tavakolpournegari¹, A. Garcia-Rodriguez¹, J. Martin¹, R. Marcos¹, A. Hernández¹

¹*Departament de Genètica i Microbiologia, Facultat de Biociències, Universitat Autònoma de Barcelona, Cerdanyola del Vallès (Barcelona), Spain.*

²*Facultad de Recursos Naturales Renovables, Universidad Arturo Prat, Iquique, Chile* Gooya.banaei@uab.cat*

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 micro and nanoplastics (MNPLs), migrate into the food, and facilitate their human ingestion. A good example is the teabags, which has become a new source of MNPLs since the traditional paper bags were substituted with “biodegradable” plastic bags. Among the different studies on the potential hazard effects of MNPLs, those related to the MNPLs released from teabags are practically inexistent. To cover this gap, we have isolated MNPLs from teabags and used a model of in vitro intestinal barrier to perform toxicological studies. The model consists of the co-culture of human gut-derived cells: enterocytes-like cells (Caco-2 and goblets-like cells (HT-29). The results from SEM-EDX and FTIR confirmed that the particles derived from teabags were polylactic acid (PLA). Moreover, using the Nano Z-sizer we could detect a hydrodynamic size of approximately 120 nm. Finally, TEM and SEM images showed the spheric shape and confirmed the size from both PLA in suspension and in the teabag tissue. Following, we aimed to study the interaction between PLA and the cells in monocultures and in differentiated co-cultures (Caco-2/HT29). Using confocal microscopy, we confirmed that PLA-NPLs could internalize into all the cells after 24 h. Moreover, NPLs were seen reaching the cell nucleus compartment. Using flow cytometry, we could quantify that PLA-NPLs had a 100% of internalization when exposing HT-29, but only 60% of internalization when exposing Caco-2 cells, suggesting that NPLs internalization may vary depending on the cell type or in vitro model. However, no significant cytotoxic effects were observed when analyzing the intracellular ROS, and the barrier integrity by TEER and LY. Therefore, this study opens new insights regarding the bio-persistence effects of MNPLs, reinforces the urgent need for multidisciplinary studies that further investigate the MNPLs as emergent contaminants, and could serve as an example for regulatory agencies to pay close attention to the regulation of MNPLs production and management.

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Polylactic acid; nanoplastics; food contaminants; gastrointestinal tract; *in vitro*.