Nanoparticles (NPs) are promising tools in medical fields, both in diagnosis and therapy (Schlorf et al., 2012; Wickline and Lanza, 2008). Despite this high applicative potential, little is known about their interaction with biological systems, almost in terms of endocytic pathways and toxicity. The first step to develop a good drug delivery systems based on NPs is to well characterize these molecular aspects. Thus, in this work, with a quantitative and qualitative approach, we studied the uptake of two representative sizes of polystyrene nanoparticles (PS-NPs), 44 nm (NP44) and 100 nm (NP100), labeled with FITC and ROD, respectively, in human adenocarcinoma gastric cells (AGS). The experiments were performed after exposure with 10µg/mL NPs for different times of incubation and temperatures (37°C and 4°C), with or without well known endocytosis inhibitor drugs (dynasore for clathrin dependent pathways and EIPA for macropinocytosis/phagocytosis). Quantitative spectrofluorimetric assays reveal a time-dependent kinetics of internalization at 37°C, with maximum values after 30 min and a decrease after 1 h for both NPs sizes. Precisely, NP44 show a high rate of uptake and a quickly internalization compared to NP100 (Fig. 1). Fluorescent images demonstrate that NPs are able to accumulate in the cytoplasm after 1 and 4 h, without reaching cell nuclei. However, NP100 tend to form aggregate after long exposition times (Fig. 3), while NP44 present an uniform cytoplasmatic distribution at all times considered (Fig. 4). Endocytosis inhibition tests show a null internalization at 4°C and a strong reduction of the uptake rate after treatment with dynasore for both NPs; EIPA, instead, partially affects NPs uptake (Fig. 2). In conclusion, in this study, we demonstrated that PS-NPs are internalized by AGS cells in a size and time dependent manner; probably, as suggest by other authors, they undergo a release process (Iversen et al., 2011). Moreover, we show that this uptake occurs through an energy dependent mechanism and that clathrin mediated endocytosis seems to be the privileged endocytic pathway for PS-NPs.

References:

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Fig. 1: Uptake kinetics of polystyrene nanoparticles in AGS cells: NP44 are faster and more efficiently internalized by AGS (1 min) compared to NP100. Both nanoparticles show the higher rate of uptake after 30 min and a release process after 1 h of incubation.

Fig. 2: Mechanism of endocytosis of polystyrene nanoparticles (PS-NPs): AGS were pre-treated with dynasore or EIPA for 30 min at 37°C or pre-incubated at 4°C for 15 min. Following pre-treatment cells were exposed to PS-NPs for 1h.

Fig. 3: Uptake of polystyrene nanoparticles 100 nm diameter labelled with Rhodamine (NP100) in AGS cells: AGS were grown in chamber slide, incubated for 4h with NP100 and analysed with epifluorescence microscopy. Nuclei were stained with Höechst. Scale bar 10 µm.

Fig. 4: Uptake of polystyrene nanoparticles 44 nm diameter labelled with fluorescein isothiocyanate (NP44) in AGS cells: AGS were grown in chamber slide, incubated 1h with NP44 and analysed with epifluorescence microscopy. Nuclei were stained with Höechst. Scale bar 10µm.