INTRODUCTION

Accumulation of senescent cells occurs in several diseases, including cancer, idiopathic pulmonary fibrosis (IPF) or type 2 diabetes. Thus, antisenescent therapies emerge as a novel therapeutic strategy. Mesoporous Silica Nanoparticles (MSNs) functionalized with galacto-oligosaccharides (GOS) have already been successfully used to target senescent cells, taking profit of their increased β-galactosidase activity (Figure 1). However, this phenotypic characteristic alone may not totally explain the specificity of the targeting. Instead, the existence of an increased number of specific galactoside receptors in cell surface is hypothesized in this work. To test this, the internalization of GOS-gated silica nanoparticles in a cellular model of senescence will be studied, with previous blockage of those receptors by addition of lactose (galactose-containing disaccharide).

SYNTHESIS AND CHARACTERIZATION OF GALACTO-OLIGOSACCHARIDE-GATED SILICA NANOPARTICLES

MSNs were used as the inorganic scaffold, loaded with rhodamine B and functionalized with the oligosaccharide Galactan (six galactose monomers), synthesized as previously described. The obtained solid, S1-GAL, was characterized by standard techniques (Figures 2-4).

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Figure 2. XRD patterns of MCM-41 as-synthesized a), calcinated MCM-41 b) and S1-GAL c).

Figure 3. TEM images of MCM-41 as-synthesized a), MCM-41 calcinated b) and S1-GAL c).

Figure 4. N2 adsorption-desorption isotherms of calcinated MCM-41 a) and S1-GAL b).

Rhodamine B content on S1-GAL amounts to 0.22 mmol/g nanoparticle.

The synthesized solid shows the structural properties characteristic of this kind of materials and a proper loading.

IN VITRO RELEASE STUDIES

To validate functionality of S1-GAL release studies were performed in the presence and absence of β-galactosidase, an enzyme able to hydrolyse the molecular gate of S1-GAL (Figure 5).

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Figure 5. Rhodamine B release in presence a) and absence b) of β-galactosidase.

CELL ASSAYS

The model for cellular senescence was ITM cells (human pulmonary fibroblasts). ITM are transduced with a fusion of the oncogene MEK1 and a modified hormone-binding domain of the human oestrogen receptor. Upon addition of 4-hydroxytamoxifen (4OHT) an oncogene-induced senescence (OIS) response is triggered.

ITM cells were incubated for 2 hours with lactose and then for 6 hours with S1-GAL (Figure 6).

Figure 6. Rhodamine B fluorescence is 3-fold higher in senescent cells. Upon addition of lactose, no difference between S1-GAL internalization in senescent and normal cells is observed, consistent with the present hypothesis.

ACKNOWLEDGEMENTS

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REFERENCES