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SELF-ASSEMBLED MANNAN NANOGEL: CYTOCOMPATIBILITY AND INTERNALIZATION BY MACROPHAGES

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Confocal colocalization, cytocompatibility, uptake, mannan nanogel.

Self-assembled mannan nanogel was synthesized and characterized with the purpose to obtain a potential therapeutic and prophylactic vaccine adjuvant and delivery system, targeting mannose receptors on surface of antigen-presenting cells.
Amphiphilic mannan, produced by the Michael addition of hydrophobic 1-hexadecanethiol (C_{16}) to vinyl methacrylated mannan (mannan-VMA), self-assembles in aqueous medium through hydrophobic interactions among alkyl chains. The particles of the mannan nanogel are stable, spherical, polydisperse, with mean hydrodynamic diameter or z-average ranging between 50 and 140 nm and with nearly neutral negative surface charge or zeta potential (Ferreira et al. 2011). According to the polymer degree of substitution (DS), defined as the percentage of grafted acrylate groups (DS_{VMA}) or alkyl chains (DS_{C16}) relative to the mannose residues, samples of mannan nanogel were named as MVC_{16}-DS_{VMA}-DS_{C16}. Mannan nanogel with different DS_{C16} and DS_{VMA}, corresponding to different nanogel size (Table 1), were selected to study the cytocompatibility of the nanogel in mouse embryo fibroblast cell line 3T3 and mouse bone marrow-derived macrophages (BMDM), using the CellTiter 96® AQueous one-solution cell proliferation assay (MTS; Promega), lactate dehydrogenase (LDH) cytotoxicity detection kitPLUS (Roche), and LIVE/DEAD® viability/cytotoxicity kit for mammalian cells (Invitrogen), according to manufacturer instructions.

Table 1. Size and zeta potential measurements obtained in dynamic light scattering (DLS) for mannan nanogel at 1 mg/mL in PBS (mean ± S.D., n = 10)

	MVC_{16}-25-11	MVC_{16}-25-22	MVC_{16}-31-20
Z-average (nm)	50.7 ± 0.9	56.4 ± 1.5	109.0 ± 2.9
Polydispersity Index (PdI)	0.589 ± 0.010	0.431 ± 0.010	0.431 ± 0.056
Zeta potential (mV)	-8.49 ± 1.71	-10.49 ± 3.76	-7.29 ± 0.37

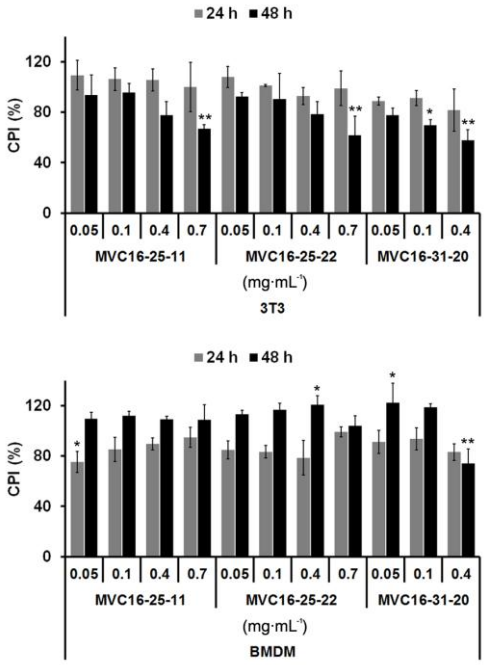

Figure 1. Effect of mannan nanogel, at the indicated concentrations, in mouse embryo fibroblast 3T3 cells and BMDM, assessed with the MTS assay. Results correspond to the mean ± S.D. of the cell proliferation index CPI (%) (* $p < 0.05$ and ** $p < 0.01$), obtained for the different groups at 24 h and 48 h incubation with mannan nanogel at the indicated concentrations. The results shown are from one experiment, representative of three independent experiments performed in triplicate.

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Genotoxicity was evaluated with comet assay (Singh et al. 1988). Statistical significance of the results was determined by one-way analysis of variance (ANOVA) with Dunnett's post-test using GraphPad Prism version 4.00. No cytotoxicity of mannan nanogel is detected up to about 0.4 mg/mL in mouse embryo fibroblast cell line 3T3 and mouse BMDM using MTS (Figure 1), LDH and LIVE/DEAD assays (data not shown). Comet assay, under the tested conditions, reveals no DNA damage in fibroblasts but possible in BMDM (unpublished results).

Uptake of mannan nanogel labeled with a fluorochrome probe (SAMSA fluorescein) by the BMDM was studied by confocal laser scanning microscopy. BMDM internalize the mannan nanogel, which is observed in vesicles in the cytoplasm (Figures 2 and 3).

Confocal colocalization image analysis denotes that the entrance and exit of nanogel and FM 4-64 might occur by the same processes – endocytosis and exocytosis – in BMDM (Figure 3). Confocal microscopy uptake inhibition analysis of mannan nanogel (0.1 mg/mL) by mouse BMDM, using pre-treatment and coincubation (1 h) with chemical inhibitors for internalization and intracellular trafficking pathways, unraveled that several distinct endocytic pathways, such as mannose receptor mediated phagocytosis and clathrin-mediated endocytosis but not caveolae-mediated endocytosis are involved in the internalization mechanism. A time-, dose- and energy-dependent uptake profile of the mannan nanogel is observed (unpublished results).

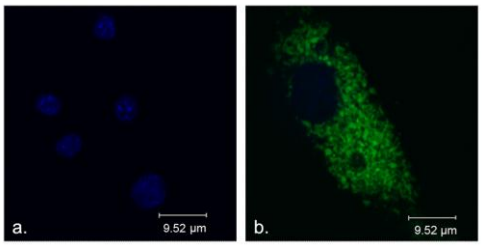

Figure 2. Confocal images of BMDM incubated 6 h without (a) and with (b) mannan nanogel labeled with SAMSA fluorescein at 0.1 mg/mL (green fluorescence). Nuclei of fixed cells are stained with DAPI (blue fluorescence). Images correspond to a central Z-stack of a representative experiment preformed in duplicate.

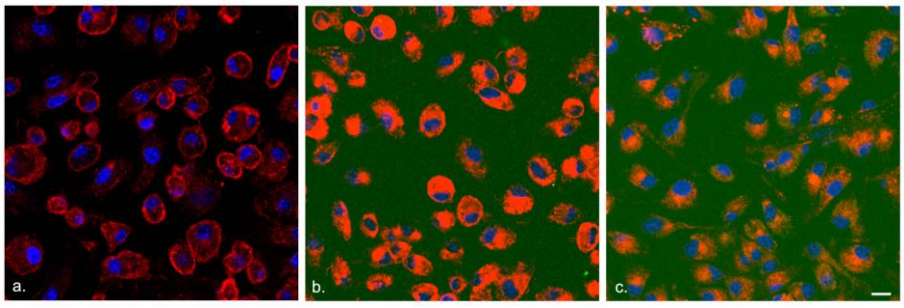

Figure 3. Confocal microscopy analysis of entrance and exit of mannan nanogel using live mouse BMDM cells. Confocal images at a certain Z-stack (scale bar = 10 µm) of a representative experiment of three independent experiments: (a) control cells labeled with DAPI (blue fluorescence) and FM 4-64 (red fluorescence); (b) cells after 3 h of incubation with the nanogel at 0.1 mg/mL (green fluorescence) and FM 4-64 present in culture medium; (c) cells after 1 h of incubation in fresh culture medium. Propidium iodide was used to screen the viability of the cells.

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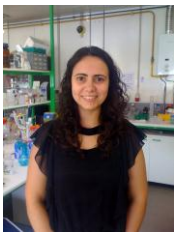

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