Materials Proceedings

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Proceeding Paper

Biocompatibility and Cellular Uptake of Fluorescent Chitosan Nanohydrogels in Murine Macrophages and B Lymphocytes †

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Abstract: Due to their intrinsic viscosity and hydrophilicity, nanohydrogel systems are used to significantly increase the efficiency of commercial contrast agents for MRI and thus effectively improve the sensitivity of the MRI technique. Since chitosan (CS) is a biocompatible polysaccharide frequently used in biomedical applications, we aimed to prepare chitosan nanohydrogels (NGs) by ionic gelation, the polysaccharide being further grafted with rhodamine (RBITC) and fluorescein isothiocyanate (FITC). In this way, the cytotoxic effect of different concentrations (5, 15, 30, 60, and 120 μg/mL) of the fluorescent CS-FITC and CS-RBITC NGs was investigated by assessing the plasma membrane integrity and the metabolic activity of RAW 264.7 murine macrophages and A20 mouse lymphoma B cells following exposure for 6 and 24 h. The cell viability (MTT assay) and lactate dehydrogenase activity were analyzed by spectrophotometric methods, while cellular uptake was observed by fluorescence microscopy. Our results showed that the exposure to CS-FITC and CS-RBITC NGs for 6 and 24 h did not induce significant changes to RAW 264.7 and A20 cells compared to control, proving a good nanogel biocompatibility for both cell lines. In addition, the fluorescence microscopy showed that cellular uptake was quite rapid and efficient for the NGs tested. Taking all of these into consideration, we can conclude that all types of nanohydrogels were biocompatible, being internalized in both cell types with predominantly cytoplasmic localization.

Keywords: nanohydrogels; cellular uptake; murine macrophages; chitosan; B lymphocytes

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1. Introduction

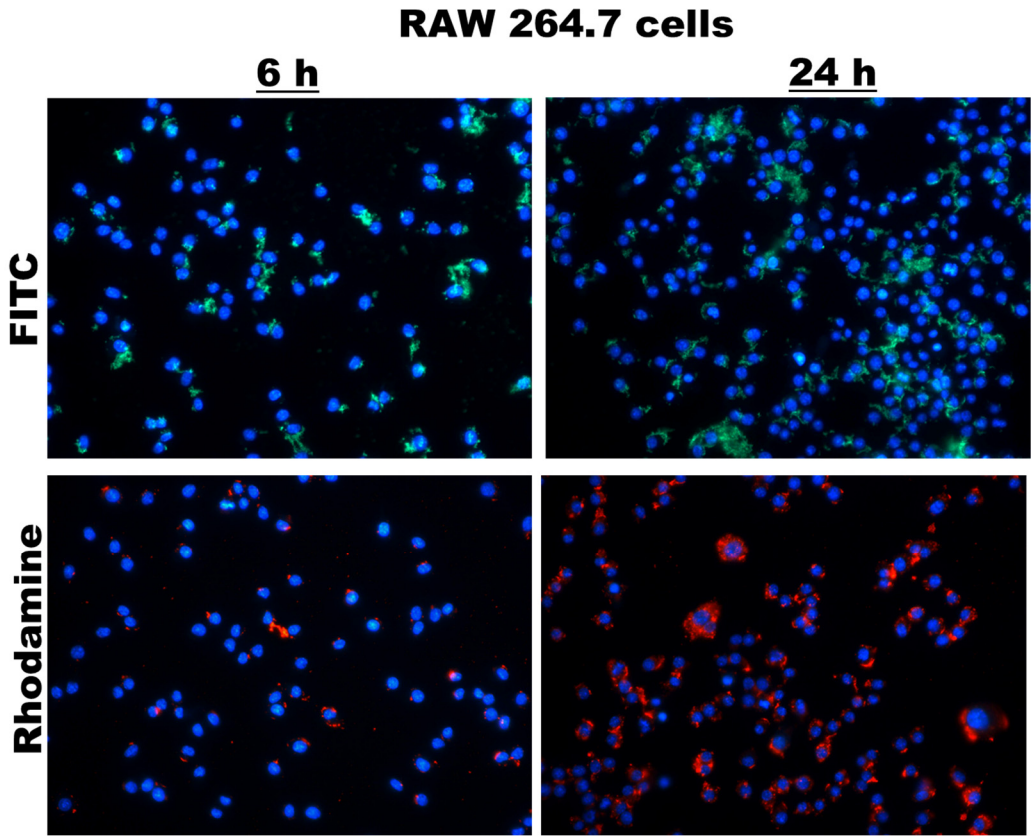
Due to their intrinsic viscosity and hydrophilicity, nanohydrogel systems are used to significantly increase the efficiency of commercial contrast agents for MRI and thus effectively improve the sensitivity of the MRI technique. Since chitosan (CS) is a biocompatible polysaccharide frequently used in biomedical applications, we aimed to prepare chitosan nanohydrogels (NGs) by ionic gelation, the polysaccharide being further grafted with rhodamine (RBITC) and fluorescein isothiocyanate (FITC).

2. Materials and Methods

The cytotoxic effect of different concentrations (5, 15, 30, 60, and 120 μg/mL) of the fluorescent CS-FITC and CS-RBITC NGs was investigated by assessing the plasma membrane integrity and the metabolic activity of RAW 264.7 murine macrophages and A20 mouse lymphoma B cells following exposure for 6 and 24 h.

3. Results

The cell viability (MTT assay) and lactate dehydrogenase activity were analyzed by spectrophotometric methods, while cellular uptake was observed by fluorescence microscopy. Our results showed that the exposure to CS-FITC and CS-RBITC NGs for 6 and 24 h did not induce significant changes to RAW 264.7 and A20 cells compared to control, proving a good nanogel biocompatibility for both cell lines. In addition, the fluorescence microscopy showed that cellular uptake was quite rapid and efficient for the NGs tested (Figure 1).



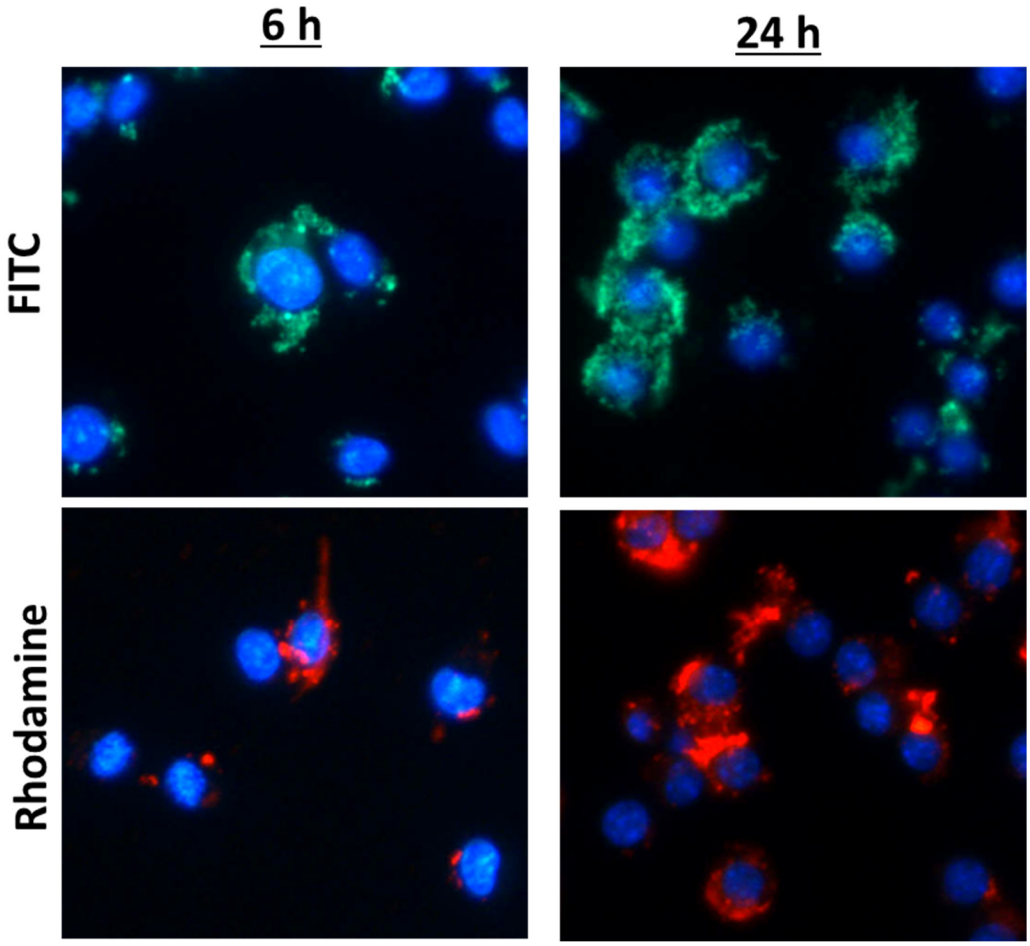


Figure 1. Fluorescence images showing the nuclei of RAW 264.7 cells stained in blue with DAPI solution and the nanogels having rhodamine or fluorescein isothiocyanate (FITC) molecules grafted on chitosan backbone.

4. Conclusions

Taking all of these into consideration, we can conclude that all types of nanohydrogels were biocompatible, being internalized in both cell types with predominantly cytoplasmic localization.

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