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Functionalized carbon nanodots interaction with human immune cells

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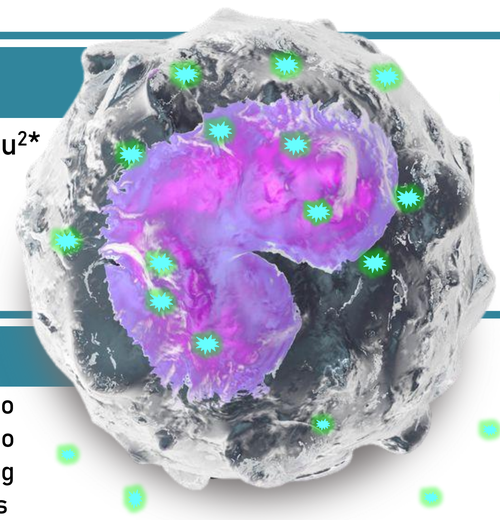
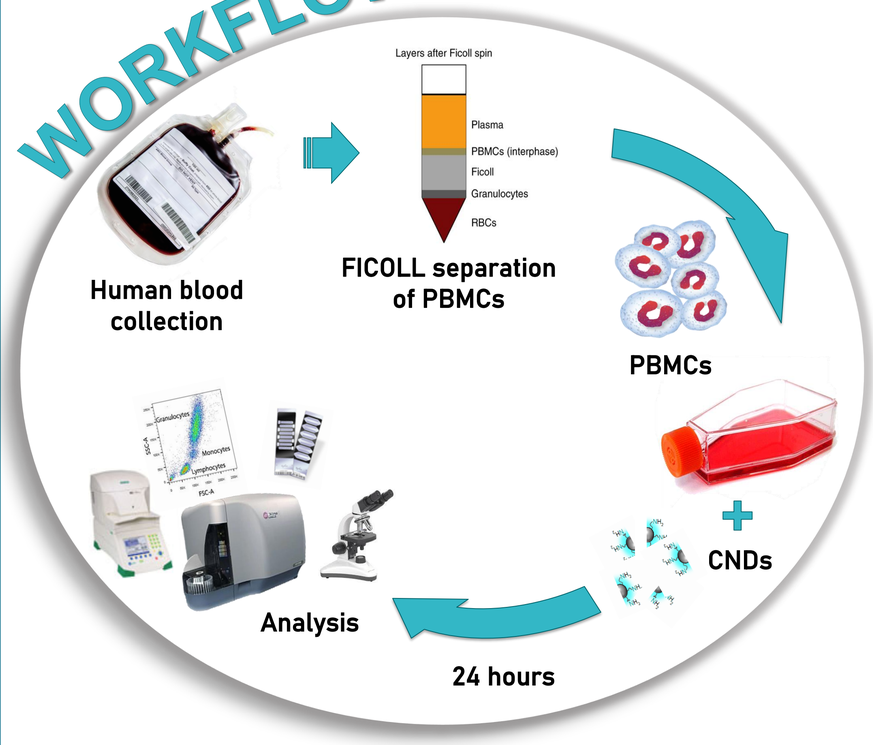


INTRODUCTION

Carbon nanodots (CNDs), quasispheroidal nanoparticles with sizes below 10 nm, are a new alternative to the popular semiconductor-based quantum dots (QDs). Compared to QDs and other organic dyes, CNDs exert superior biological properties, high aqueous solubility, chemical inertness, easy functionalization, and high resistance to photobleaching [1]. Thanks to their superior physicochemical properties, such as luminescence emission and easy functionalization [2] CNDs are emerging as promising biomedical tools. However, the application of CNDs in biomedicine requires the assessment of their impact on the complexity of the immune system. Therefore, the aim of this study was to exploit our experience, gained on the impact of nanomaterials on immune cells [3,4] to evaluate the interactions of human peripheral blood mononuclear cells (PBMCs) with six different functionalized CNDs: nitrogen-doped carbon nanodots (NCNDs), BODIPY-doped carbon nanodots (BCNDs), as well as their methylated (mNCNDs and mBCNDs, respectively) and carboxylated forms (NCNDs-COOH and BCNDs-COOH, respectively).

WORKFLOW



Effects of CNDs on cell viability

Figure 1. Effects of CNDs on human PBMC viability

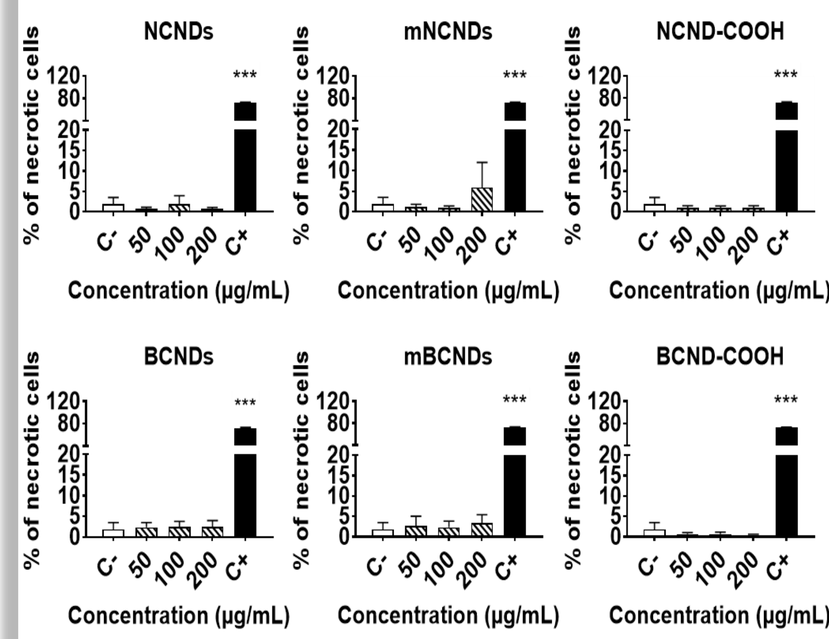
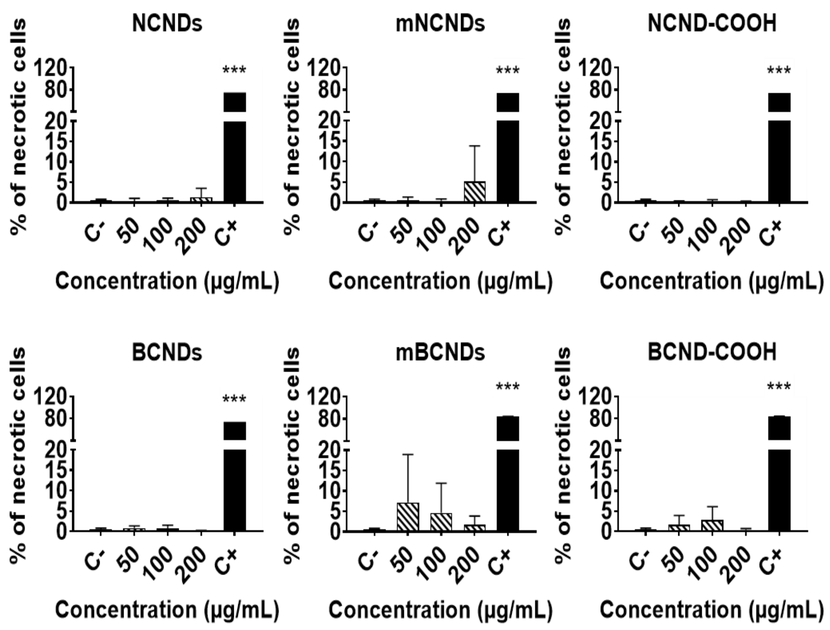


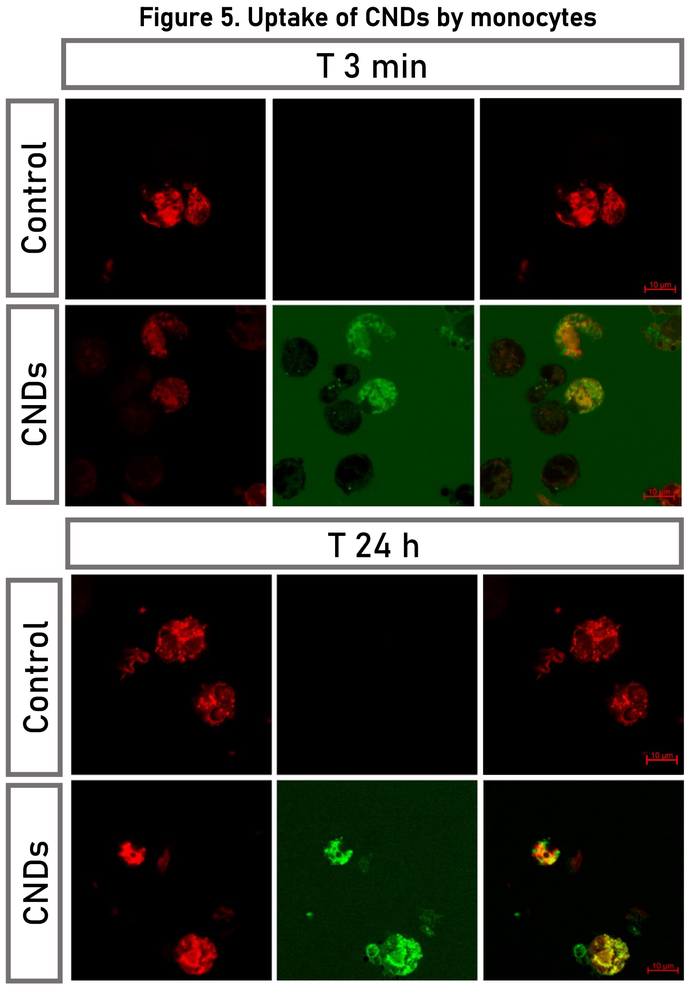
Figure 2. Effects of CNDs on human monocyte viability



The effects of different concentrations of CNDs (50, 100 and 200 µg/mL) were evaluated on peripheral blood mononuclear cells (PBMCs) viability after 24 h exposure by flow cytometry, using propidium iodide (PI) to measure necrotic cells. As shown in **figure 1**, CNDs did not induce any significant effect on cell viability, demonstrating the high biocompatibility of these materials on immune cells. On the contrary, as expected, the positive control (C+ EtOH 70% v/v) impaired cell viability by 72% (statistical differences: ***, p<0.001).
The effects were then evaluated specifically on primary human monocytes, representing the most specialized cell types in the phagocytic process and being a representative cell type for the innate response. In monocytes, CNDs did not induce any significant effect on cell viability, as shown in **figure 2**, demonstrating the high biocompatibility of these materials on this PBMC immune cell subpopulation. On the contrary, as expected, the positive control (C+ EtOH 70% v/v) impaired cell viability by 84% (***, p<0.001).

Uptake of CNDs by monocytes

Figure 5. Uptake of CNDs by monocytes



The obtained findings were confirmed evaluating the uptake of mBCNDs (200 µg/mL) in human THP-1 monocytes after 24 h exposure by confocal fluorescence microscopy (**figure 5**). Cells were stained with 1 uM Cell TraceTM Far Red stain for cell labelling (**left side**). After three minutes and 24 h exposure mBCNDs presence inside the cells was detected in the green channel (470-610 nm) (**middle side**). The acquired images demonstrated that CNDs (green) were able to be efficiently accumulated in monocytes (red) in the overlapping figures in yellow (**right side**). Images were acquired by Eclipse C1si, on an inverted microscope TE2000U, Nikon (objective 63x).

Effects of CNDs on cell uptake

Figure 3. Uptake of CNDs by human PBMCs

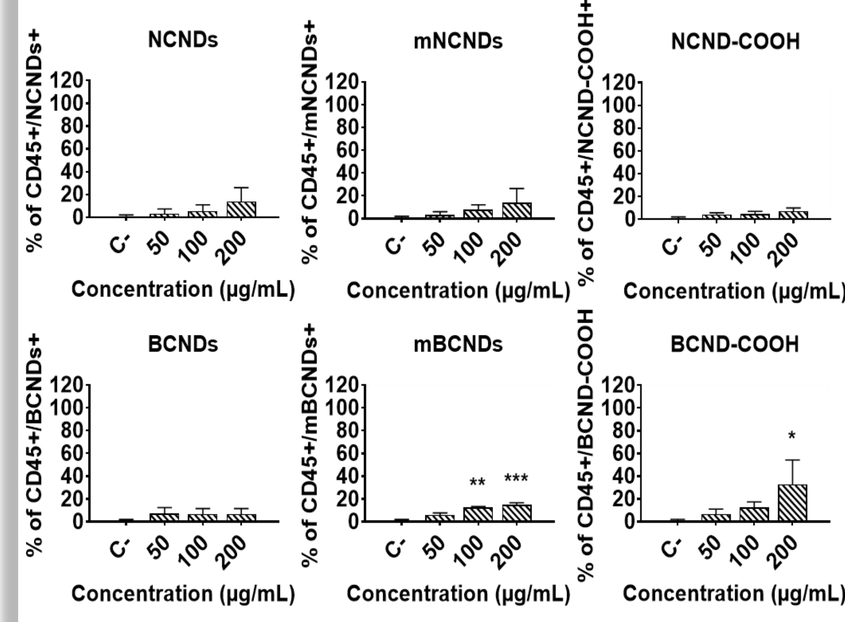
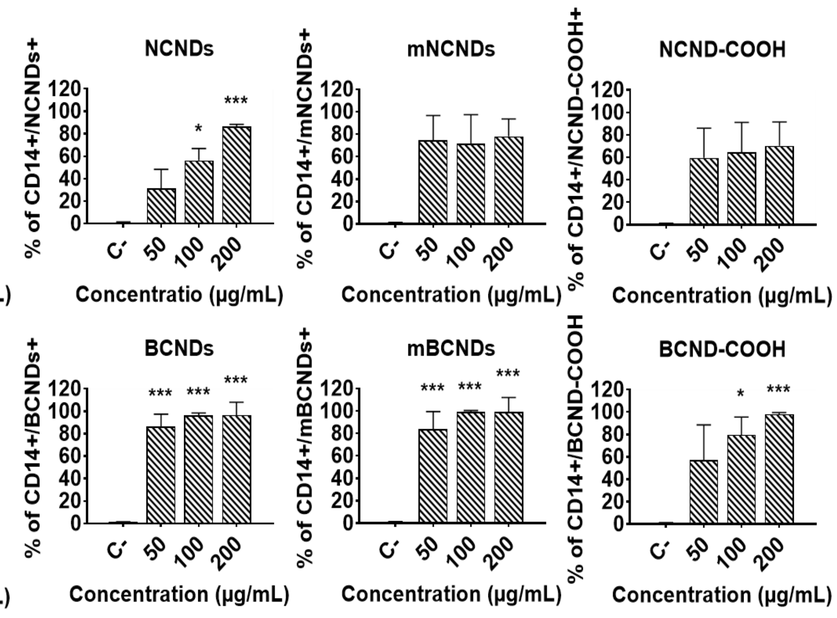


Figure 4. Uptake of CNDs by human monocytes



PBMCs were then evaluated for their ability to internalize CNDs. Cells were exposed to different concentrations of CNDs (50, 100 and 200 µg/mL) and the cell uptake was measured after 24 h by flow cytometry. As reported in **figure 3**, significant cellular uptake was observed in PBMCs for two BODIPY-doped CNDs: mBCNDs, starting from the concentration of 100 µg/mL (**, p<0.01), and BCND-COOH, only at the highest concentration tested (200 µg/mL) (*, p<0.1). The obtained findings suggest the role of the functionalization of CNDs on their uptake by PBMCs. After gating on a wide variety of human immune cells (data not shown), primary human monocytes have shown the highest degree of CNDs internalization. Results showed a concentration-dependent internalization of CNDs, particularly relevant for BODIPY-doped CNDs. As reported in **figure 4**, at the highest concentration tested (200 µg/mL), mBCNDs were the most internalized (99%), followed by BCND-COOH (98%), BCNDs (96%), NCNDs (86%), mNCNDs (78%) and NCND-COOH (70%). The obtained findings confirm the hypothesis of a role for the functionalization of CNDs on their uptake.

CONCLUSIONS

Overall, the obtained results demonstrated the high biocompatibility and concentration-dependent internalization of CNDs in human PBMCs, particularly relevant for BODIPY-doped CNDs, which was even more evident when considering monocyte subpopulation, suggesting a role for the functionalization of CNDs on their uptake and paving the way for possible future biomedical applications of these promising nanomaterials. Further analysis on the functionality of immune cells are needed in the future.

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