A semi-automated microfluidic platform for real-time tracking of cancer cells and investigation of nanoparticles cellular uptake

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Statement of Purpose: Circulating tumor cells (CTCs) have been highly studied due to its implication in cancer dissemination and metastases. Thus, an approach capable of hindering their dissemination could help prevent 90% of cancer deaths (1). Labeled nanoparticles (NPs) have been proposed to track cells and monitor the efficiency of any new cancer therapy in real time. The association of NPs to a microfluidic device, such as Vena8 biochips (Figure 2), allows the accomplishment of functions *in vitro* that are not easily imaginable in conventional biological analysis (e.g., simulation of physiological flow and shear stress). This novel approach may expedite therapies' validation and its successful translation into clinics. In this study, we aim to develop an improved platform composed of labeled dendrimer nanoparticles and a microfluidic device for real-time monitoring of cancer cells fate.

Methods: Carboxymethyl-chitosan/poly(amidoamine) (CMCht/PAMAM) dendrimer nanoparticles were synthetized (2), labeled with the fluorescent label probe Fluorescein-5(6)-isothiocyanate (FITC) and characterized using Transmission electron microscopy, Atomic force microscopy, Dynamic light scattering, and Differential scanning calorimetry. After culturing a variety of cancer cell types, including HeLa (Cervical Carcinoma), HCT-116 (Colon Carcinoma) and U87MG (Glioblastoma), in the presence of these nanoparticles, cell viability and internalization efficiency in static (standard cultures) and dynamic (microfluidic cultures) conditions were investigated by MTS/DNA and flow cytometry as well as fluorescence microscopy.

Results: Nanoparticles were characterized by several physicochemical techniques. Results suggest that the synthesis was successful and nanoparticles are round-shaped with a mean size of 50 nm and a negative charge of -34.3 ± 3 (mV) at neutral pH. Regarding cell viability in static conditions, no cytotoxic effects were observed when comparing the controls (absence of NP) and NP at 0.5 $mg.mL^{-1}$. However, different responses were observed regarding the presence of dendrimer nanoparticles when comparing static to dynamic conditions, with a tendency towards higher sensitivity when subjected to confinement (Figure 1). The results were corroborated by flow cytometry analysis (7AAD marker). Regarding internalization efficiency, higher internalization rates of the nanoparticles were observed in dynamic conditions as compared to traditional static culturing conditions, showing once again the relevance of using a dynamic system for validation of new therapies.

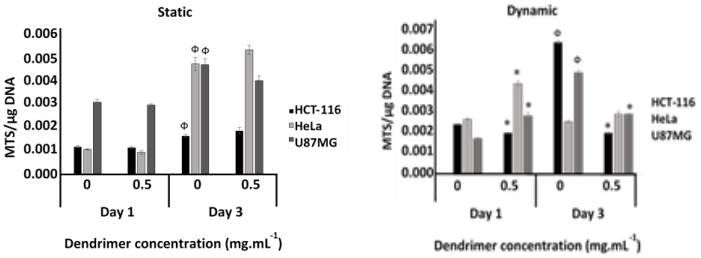

Figure 1. Cytotoxicity of CMCht/PAMAM over HCT-116, HeLa and U87MG human cancer cell lines in static and dynamic conditions. Assays were conducted to assess cytotoxicity/viability over the cells at a concentration of 0.5 $mg.ml^{-1}$ at different time points (days 1 and 3) and it in static and dynamic conditions. (* indicates significant differences when comparing 0.5 $mg.ml^{-1}$ to control (0 $mg.ml^{-1}$) at each time point. Φ indicates significant differences when comparing controls from day 3 to day 1 to determine cell proliferation).

Figure 2. Vena8 microfluidic biochip and pump (Cellix®, Irland).

Conclusions: This study provided proof-of-concept on the use of a platform composed of microfluidic chip together with fluorescence labeled dendrimer nanoparticles for the validation of new chemotherapeutic agents. In fact, the results show different responses to the presence of 0.5 $mg.mL^{-1}$ dendrimer nanoparticles when comparing static to dynamic conditions. There is a clear tendency towards higher sensitivity when subjected to confinement, flow and shear stress (dynamic conditions). Moreover, the observed high internalization rates of the nanoparticles can be beneficial, making them excellent intercellular carrier of anti-cancer drugs. Thus, the microfluidics can enable the development of diagnostics platform and personalized therapies, as it opens the possibility to a valuable system to test and validate new chemotherapeutic agents

References

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