Investigation of Dendrimer-based nanoparticles cellular uptake and cell tracking in a semi-automated microfluidic platform

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Statement of Purpose: Dendrimers can act as drug delivery systems for controlling cellular fate from inside cells by being engineered for its chemistry, bioavailability and biocompatibility [1]. Indeed, other polymeric nanoparticles have already been reported and studied as carriers for drugs, genes and image contrast agents, therefore finding many possibilities in tissue engineering and regenerative medicine [1]. In this study, synthesis and physicochemical characterization of Carboxymethyl-chitosan/poly(amidoamine) dendrimer nanoparticles (CMCht/PAMAM NP's) were performed. Also, the internalization efficiency was tested with L929 cells as well as cellular trafficking, achieved by grafting fluorescent label probe Fluorescein-5(6)-isothiocyanate (FITC), in static and dynamic conditions (Kima Pump bioreactor). A microfluidic device such as Kima Pump and Vena8 biochip is able to realize functions that are not easily imaginable in conventional biological analysis, such as highly parallel, sophisticated high-throughput analysis, single-cell analysis in a well-defined manner, and tissue engineering with the capability of manipulation at the single-cell level [2].

Methods: Carboxymethylchitosan/poly(amidoamine) dendrimer nanoparticles (CMCht/PAMAM NP's) were synthetized and labelled with fluorescein isothiocyanate (FITC) according to Oliveira *et al.* [3].

To access internalization efficiency, semi-automated microfluidic platform, Vena8 Endothelial+ biochips (Cellix®, Dublin, Ireland) was used, to mimic physiological flow conditions. Biochips were coated using a standard pipette tip and ~12 µL of fibronectin (Sigma, Germany) into each microchannel and placed at 4ºC for overnight coating. A solution of FITC-CMCht/PAMAM NP's at a concentration of 0.5 mg.mL^{-1} was prepared in a complete culture medium and then transferred to Kima Pump to initiate perfusion. Perfusion was performed for 24 hrs and 48 hrs at a flow rate of 2 µL/min for 2 min. (Period I) followed by 20 min of pause (Period II).

Results: CMCht/PAMAM NP's were successfully synthetized as shown by H^1NMR analyses. Moreover, they exhibited good physicochemical properties, with a consistent nanosphere-like shape and a diameter of ~30 nm, as shown by TEM, DLS and AFM analysis. Fluorescent-probe labeled CMCht/PAMAM NP's were found to be internalized with high efficiency, either in conventional static conditions and when seeded and grown in a biochip under perfusion with Kima Pump. The flow rate as well as the cell density has been optimized. It was found that the best flow rate was 2 µL/min (Period I), followed by a pause of 20 min. (Period II). Superior flow rates induced cell detachment and inferior rates did not support cell growth, maybe due to shortness of nutrients supplied by the media. Optimization studies suggest 70,000 cells/channel is the ideal concentration for a 48 hrs study. The use of the biochips connected to Kima Pump bioreactor permits reaching cell confluence in dynamic conditions in just 3 hours and allows the assay to start promptly. Fluorescent-labeled NP's were up-taken by L929 cells with high efficiency, either in dynamic or conventional static conditions, as shown in the images.

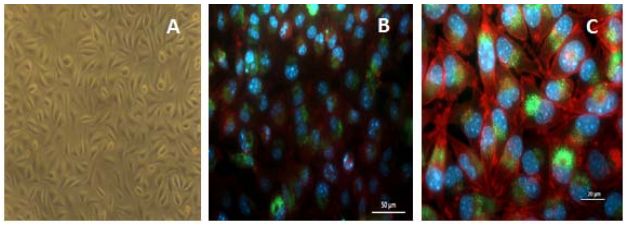

Figure 1. A) L929 cells reaching confluency 3 hrs after seeding in Vena8 biochips (Cellix®, Dublin, Ireland); B) Fluorescence microscopy images of L929 cells in the presence of FITC-labeled CMCht/PAMAM NP's 0.5 mg.mL^{-1} (green) in static conditions. C) Fluorescence microscopy images of L929 cells in the presence of FITC-labeled CMCht/PAMAM NP's 0.5 mg.mL^{-1} (green) in dynamic conditions using KimaPump (Cellix®)

Conclusions: CMCht/PAMAM NP's were successfully synthetized and exhibit good physicochemical properties, with a consistent nanosphere-like shape and a diameter around 30 nm. Fluorescent probe labeled nanoparticles were found to be internalized with high efficiency in L929 cell line, either in conventional static conditions and when seeded and grown in a biochip under perfusion with Kima Pump, with a flow rate of 2 µl/min, consisting in a faster way to perform assays with lower consumption of reagents and materials. This experiment is therefore a proof-of-concept and will allow the design of other experiments in a faster way, as it is compatible with fluorescence microscope and cells can be recovered from the biochip after the experiment. It is our particular interest to engineer the CMCht/PAMAM NPs for applications in the intracellular controlled delivery of biological agents, cell tracking and differentiation, together with the optimization of the bioreactor and biochips to create new models of disease.

References:

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