Universidade do Minho
Escola de Engenharia

Semana da Escola de Engenharia
October 24 - 27, 2011

MULTIFUNCTIONALIZED CMCHT/PAMAM DENDRIMER NANOPARTICLES MODULATE THE CELLULAR UPTAKE IN PRIMARY CULTURES OF GLIAL CELLS

Susana R. Cerqueira,[1,2,3] Bárbara L. Silva,[2,3] Joaquim M. Oliveira, [1,3] João F. Mano, [1,3] Nuno Sousa,[2,3] António J. Salgado,[2,3] Rui L. Reis[2,3]

[1]3B´s Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, S. Cláudio do Barco, 4806-909 Caldas das Taipas, Guimarães

[2]Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, 4710-057 Braga, Portugal

[3]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

E-mail: susana.cerqueira@dep.uminho.pt

KEYWORDS
CD11b antibody; dendrimers; glial cells; nanoparticles

ABSTRACT

The efficiency of the treatments involving CNS disorders is commonly diminished by the toxicity, reduced stability and lack of targeting of the administered neuroactive compounds We have successfully multifunctionalized CMCht/PAMAM dendrimer nanoparticles with the CD11b antibody. The modification of the new antibody-conjugated nanoparticles was confirmed by S-TEM observation and 1H NMR and FTIR spectroscopy. Cytotoxicity assays revealed that the conjugates did not affect the viability of both primary cultures of glial and microglial cells. Trace analyses of FITC-labelled nanoparticles revealed that the uptake of antibody-conjugated nanoparticles was conserved in microglial cells but significantly decreased in astrocytes and oligodendrocytes.

INTRODUCTION

A major issue for drug delivery in the CNS is the presence of biological barriers such as the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (B-CSF) that can obstruct the entry of therapeutic molecules to the nervous tissues. However, in certain conditions the integrity of the barriers is disrupted, opening a 'window of opportunity' for the entry of drugs that can limit the extent of following potential secondary damage. Still, efficient drug delivery to the target cells remains a crucial task for the success of these therapies. The use of nanoparticle-based systems presents advantageous features, namely for cell or tissue targeting. A new surface engineered macromolecular carrier was recently proposed to be used in CNS applications, showing high internalization levels and no significant cytotoxicity over neurons and glial cells.[1,2] Therefore, the aim of this study was to achieve a higher functionalization level of the CMCht/PAMAM dendrimer nanoparticles by conjugating to the latter a targeting ligand, examining a possible modulation of their internalization behaviour in glial cells.

MATERIALS AND METHODS

Nanoparticles synthesis and characterization

CMCht/PAMAM nanoparticles (NPs) were produced as already described elsewhere.[1] Fluorescein isothiocyanate (FITC) was covalently conjugated to the nanoparticles after incorporation of methylprednisolone by a precipitation route. In order to conjugate the CD11b antibody, a crosslinking reaction via EDC was performed. The final solutions were frozen at -80ºC and subsequently freeze-dried (Telstar-Cryodos-80, Spain). Fourier transform infrared spectroscopy (FTIR) and proton nuclear magnetic resonance (^{1}H NMR) were performed, as well as scanning-transmission electron microscopy (S-TEM).

In vitro tests

Primary cultures of glial cells were established isolating them from newborn P4 Wistar rats. MTS test was used to assess the viability of the cells one week after the NPs addition. Immunocytochemistry was performed in the cultures using the corresponding primary antibody (GFAP: astrocytes; O4: oligodendrocytes). The cells were observed with a Fluorescence Microscope.

Semana da Escola de Engenharia
October 24 - 27, 2011

RESULTS AND DISCUSSION

The multifunctional modification of the CMCht/PAMAM dendrimer nanoparticles was confirmed by Fourier transform infrared spectroscopy (FTIR) and proton nuclear magnetic resonance (1H NMR). From Figure 1, it is visible both the FTIR and the ^{1}H NMR spectra of CD11b antibody-conjugated CMCht/PAMAM dendrimer nanoparticles revealed new signals which indicate that the conjugation actually occurred. STEM analysis also confirmed the spheric morphology of the nanoparticles. Regarding the biological assays, no negative effect in the normal cell activity was observed (**Figure 2**). Also, the CD11b conjugation to the MP-loaded CMCht/PAMAM dendrimer nanoparticles did not alter the percentage of microglial cells internalizing the nanoparticles (Figure 3). Importantly, however, in astrocytes and oligodendrocytes, a drastic and significant decrease in the antibody-conjugated nanoparticles uptake was observed. Thus, CD11b antibody-conjugated nanoparticles contributed to the improvement of their intracellular uptake, conferring a targeted profile to the system, drastically reducing the uptake by astrocytes and oligodendrocytes.

CONCLUSIONS

Within the present study it was possible to demonstrate a new nanoparticles uptake profile by astrocytes and oligodendrocytes with the new modified nanoparticles. These cells significantly reduced the levels of CD11b antibody-conjugated nanoparticles internalization, while maintaining maximum uptake by microglial cells.

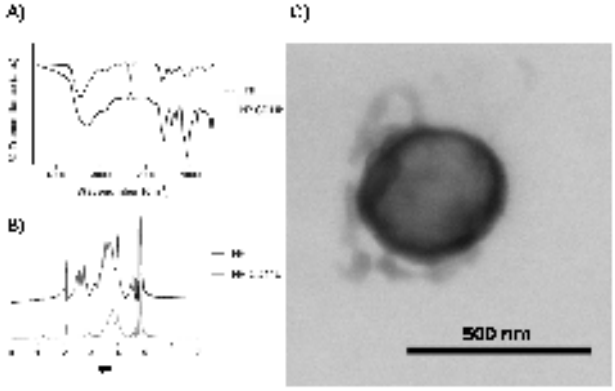

Figure 1: Characterization of the multifunctional NPs.

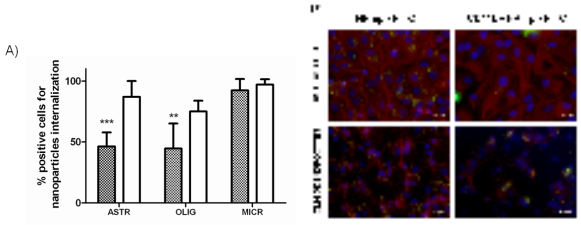

Figure 2: Cell uptake by primary glial cell cultures.

REFERENCES

[1] Oliveira, J.M *et al*. 2008, "Surface Engineered Carboxymethylchitosan/Poly(amidoamine) Dendrimer Nanoparticles for Intracellular Targeting" *Advanced Functional Materials*, 18, 1840.

[2] Salgado, A.J *et al*. 2010, "Carboxymethylchitosan/Poly(amidoamine) Dendrimer Nanoparticles in Central Nervous Systems-Regenerative Medicine: Effects on Neuron/Glial Cell Viability and Internalization Efficiency" *Macromolecular Bioscience*, 10, 1130.

AUTHORS' BIOGRAPHIES

Susana R. Cerqueira was born in Braga. She graduated from Applied Biology in Minho University in 2008. During the same year she enrolled in a project entitled "Cell Targeted Nano-Based Drug Delivery Systems for Tissue Engineering and Medicine Applications" of the 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, in collaboration with the Life and Health Sciences Institute (ICVS), both from Minho University. She is now working on her PhD project focused on drug delivery nano-devices specifically directed to nervous cells, focusing on Spinal Cord Injury therapies.