Synthesis and characterization of a novel nanomicellar system Pluronic-PEI suitable for gene and drug co-delivery in cancer therapy

Cátia Domingues 1,2,3, Ivana Jarak 1, Carla Varela 1,4,5, Elisiário Tavares 1,4,5, Fernanda Roleira 1,4,5, Carmen Alvarez-Lorenzo 6, Angel Concheiro 6, Francisco Veiga 1,2, Marília Dourado 3,9,10, Ana Figueiras 1,2, *

Abstract: Polyethylenimine (PEI) is a synthetic cationic polymer recognized as a non-viral gene carrier with high transfection efficiency [1]. However, cytotoxicity issues limit its use. Pluronic block-copolymers conjugated with PEI have demonstrated promising results for multiparametric target gene/drug co-delivery in cancer with reduced side-effects [1,2].

The goal of this work was to synthesize and characterize a novel nanosystem Pluronic L121-PEI for gene/drug co-delivery.

For this purpose, hydroxyl groups from Pluronic were activated using acryloyl chloride leading to the synthesis of a diacrylate intermediate which was further conjugated with PEI. FTIR and 1H-NMR spectroscopy were used for structural characterization. Particle size, polydispersity index (PDI) and zeta potential were assessed by Dynamic and Electrophoretic Light Scattering, respectively. A fluorescent pyrene probe was used to evaluate the Critical Micellar Concentration (CMC). Hemolysis experiment was performed to estimate the in vitro biocompatibility of the nanosystem.
FTIR analysis showed that pluronic diacrylate was successfully synthetized as a new band around 1730 cm$^{-1}$ (C=O bond) appears. Its conjugation with PEI was confirmed by the presence of a band between 3380-3390 cm$^{-1}$ (N-H bond). 1H-NMR results showed characteristic proton peaks from Pluronic (-CH3 at δ1.1 ppm) and from PEI (-CH2-CH2NH- between δ2.7–3.4 ppm) and the molar ratio Pluronic-PEI was 1:2. Nanoparticles hydrodynamic diameter was ca. 125 nm with a PDI below 0.250, and a charge nearby +30 mV. The CMC was around 50 μg/mL. The hemolysis ratio of a 5 mg/mL nanomicellar solution was less than 5%.

A novel Pluronic L121-PEI was successfully synthesized which is able to self-assemble in aqueous solution leading to the formation of biocompatible cationic polymeric micelles. Their small size is suitable for tumor-targeting and as they are positively charged they can be also valuable for gene delivery. Overall, this new nanosystem could be a promising multiparametric nanoapproach for gene/drug co-delivery in cancer therapy.

**Keywords:** cancer therapy; gene/drug co-delivery; Pluronic L121; Polyethyleneimine; Nanosystems

**Funding:** This work received financial support from National Funds (FCT/MEC, Fundação para a Ciência e Tecnologia/Ministério da Educação e Ciência) through the project UID/QUI/50006/2013, co-financed by European Union (FEDER under the Partnership Agreement PT2020). It was also supported by the grant FCT PTDC/BTM-MAT/30255/2017 (POCI-01-0145-FEDER-030255) from the Portuguese Foundation for Science and Technology (FCT) and the European Community Fund (FEDER) through the COMPETE2020 program.

**References**


© 2020 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license (http://creativecommons.org/licenses/by/4.0/).