IECP 2020

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pharmaceutics

MDPI

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FFUC FACULDADE DE FARMÁCIA UNIVERSIDADE DE COIMBRA

FMUC FACULDADE DE MEDICINA UNIVERSIDADE DE COIMBRA

LAQV requimte LABORATÓRIO ASSOCIADO PARA A QUÍMICA VERDE

CIEPQPF CIMAGO

Abstract

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 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 confirmed that pluronic was successfully conjugated with PEI as a band between 3380-3390 cm−1 (N-H bond) was observed. 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). 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%. Overall, a novel Pluronic L121-PEI was successfully synthesized and could constitute a promising multiparametric nanoapproach for gene/drug co-delivery in cancer therapy.

Keywords: cancer; polymeric micelles; micelleplexes; Pluronic L121; polyethyleneimine (PEI)

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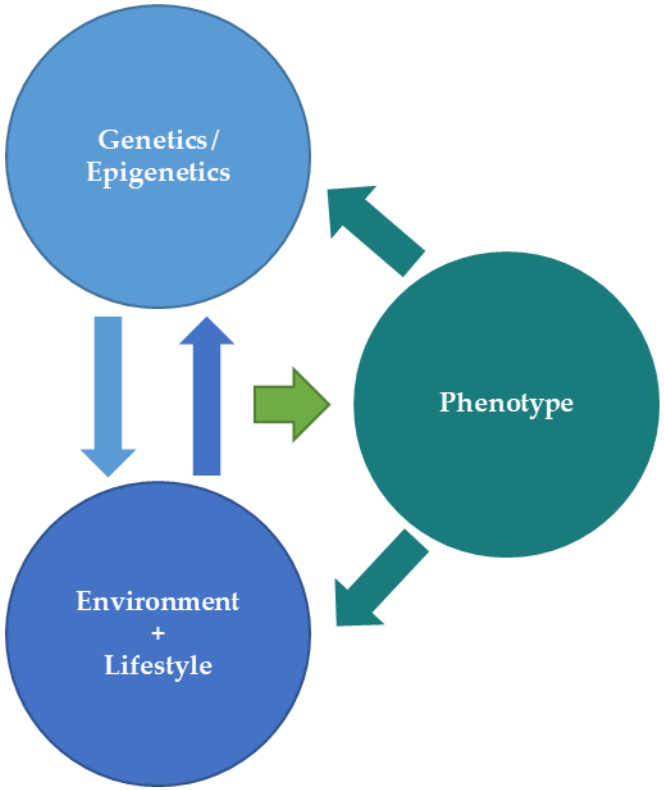
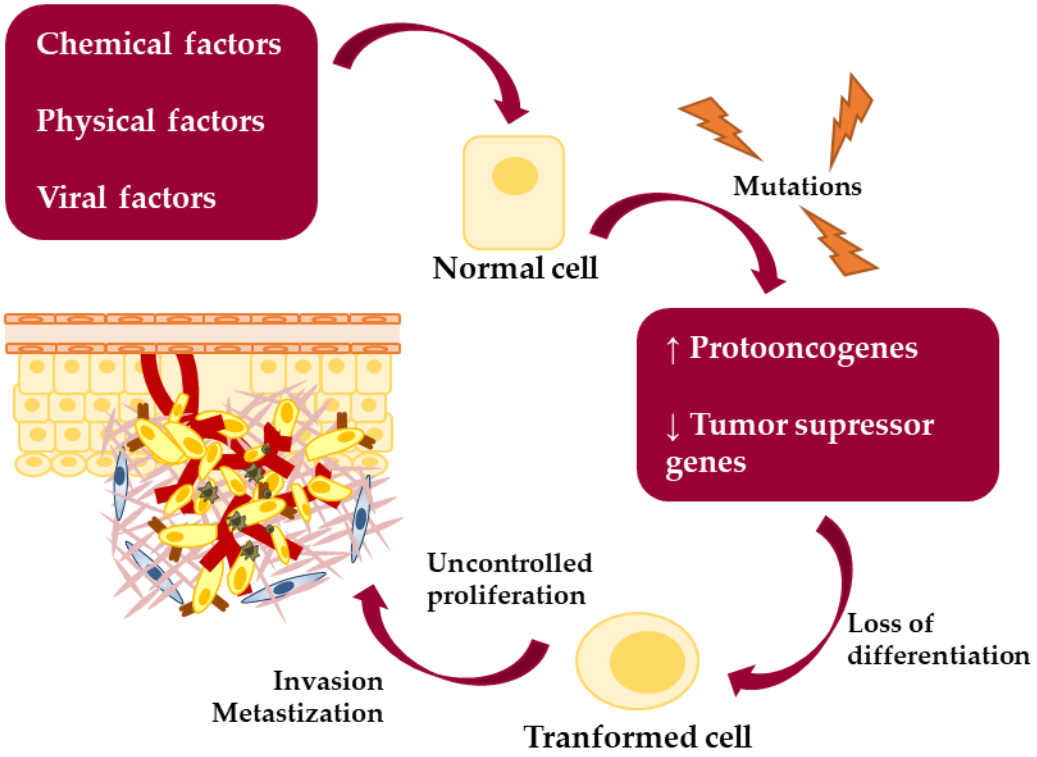
Background
Cancer





- Cancer is a multistep disease that results from the crosstalk between genetic/epigenetic alterations and environmental/lifestyle factors;
- Despite the advances in diagnosis and treatment, recurrences, resistances to the treatments and adverse side effects are frequent.

Domingues C. *et al*, 2019

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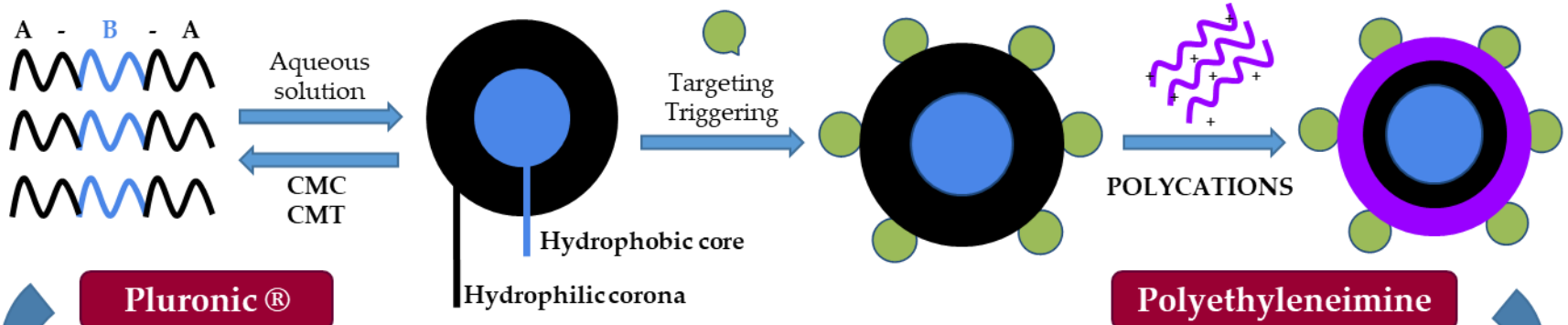
Background

Micelleplexes advantages as nanocarriers for cancer



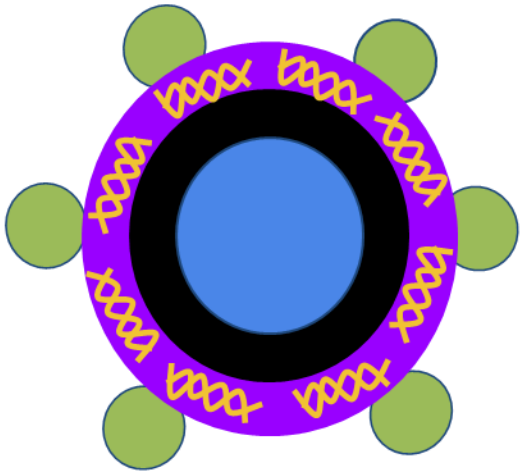
Pluronic ®

- Non-ionic surfactants with amphiphilic structure which promotes the formation of POLYMERIC MICELLES in aqueous solution above their critical micellar concentration and temperature;
- Small size allows tissue penetration, passive tumor targeting and avoids the RES and macrophages recognition, resulting high blood circulation time;
- Present high drug load capacity;
- EPR effect;

Polyethyleneimine

- Protonable amine groups promote electrostatic interaction with negatively charged phosphate groups of genetic material;
- "Proton sponge" effect which avoid endosomal capture.
- High *in vitro* and *in vivo* transfection efficacy;

GENETIC MATERIAL



Multifunctional Micelleplexes

Domingues C. *et al*, 2018
Domingues C. *et al*, 2019

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Aim

To synthesize and characterize a novel nanosystem Pluronic L121-PEI for gene/drug co-delivery.

Experimental Design

1st Task – Synthesis of Pluronic L121-PEI

2nd Task – Structural and physicochemical characterization of the new synthetized nanosystem

- Structural analysis: FTIR (Fourier Transform Infrared Spectroscopy)
- ^{1}H-NMR (Nuclear Magnetic Resonance)
- DLS (Dynamic Light Scattering)
- ELS (Electrophoretic Light Scattering)
- Pyrene fluorescente probe

3rd Task – *In vitro* biocompatibility of the new synthetized nanosystem

- Hemolysis test

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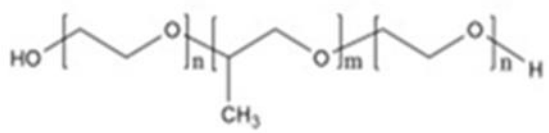
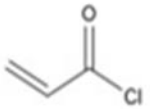
Synthesis strategy of Pluronic L121-PEI



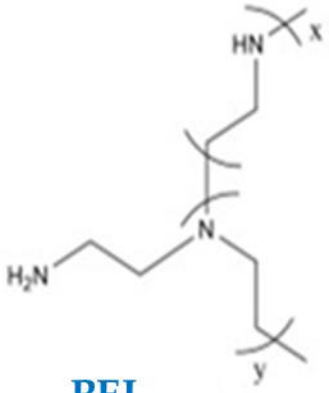
Pluronic

Reflux

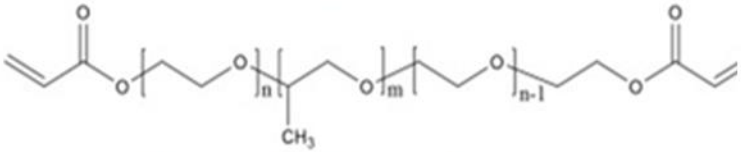
Organic solvent removal



Acryloyl chloride



PEI



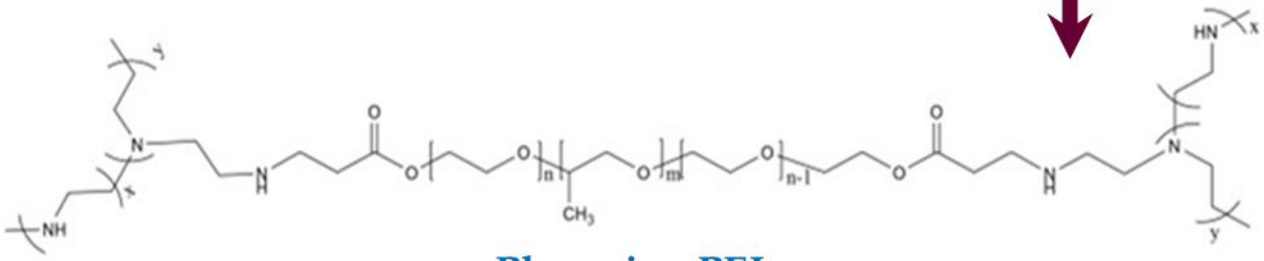
Pluronic Diacrylate

Reflux

Organic solvent removal

Purification step

Freeze drying step



Pluronic - PEI

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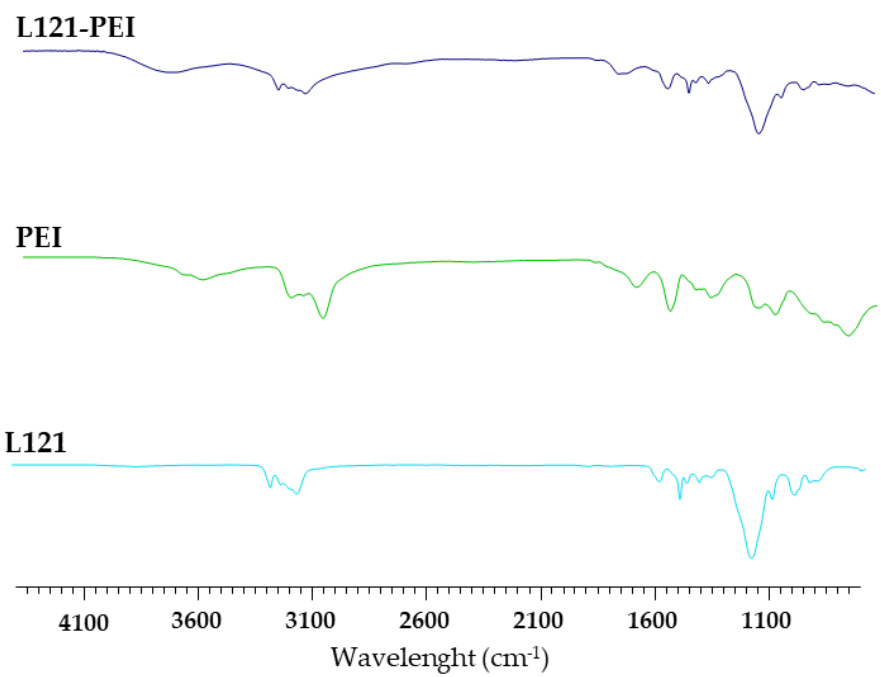
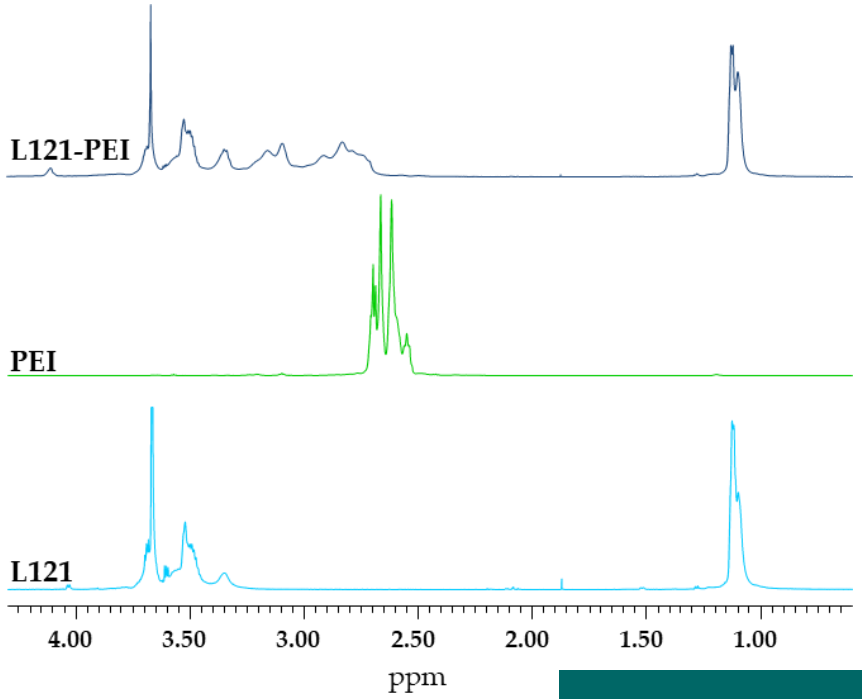
Results

Structural Analysis

FTIR Spectroscopy



^{1}H-NMR Spectroscopy



- **FTIR analysis confirmed the conjugation of the activated pluronic with PEI by the presence of a band between 3380-3390 cm^{-1} (N-H bond);**
- **^{1}H-NMR results showed characteristic proton peaks from Pluronic (-CH_3 at δ1.1 ppm) and from PEI (-CH_2-CH_2NH- between δ2.7–3.4 ppm).**

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Results

Physicochemical characterization

Hydrodynamic diameter, Polydispersity index and Zeta Potential

(Dynamic light scattering and Electrophoretic light scattering)

Table I – Average size, polydispersity index and zeta potential of different Pluronic L121-PEI nanosystems.

	Size (nm)	Polydispersity Index	Zeta Potential (mV)
L121-PEI	125.2 ± 2.6	0.165 ± 0.020	27.8 ± 1.270

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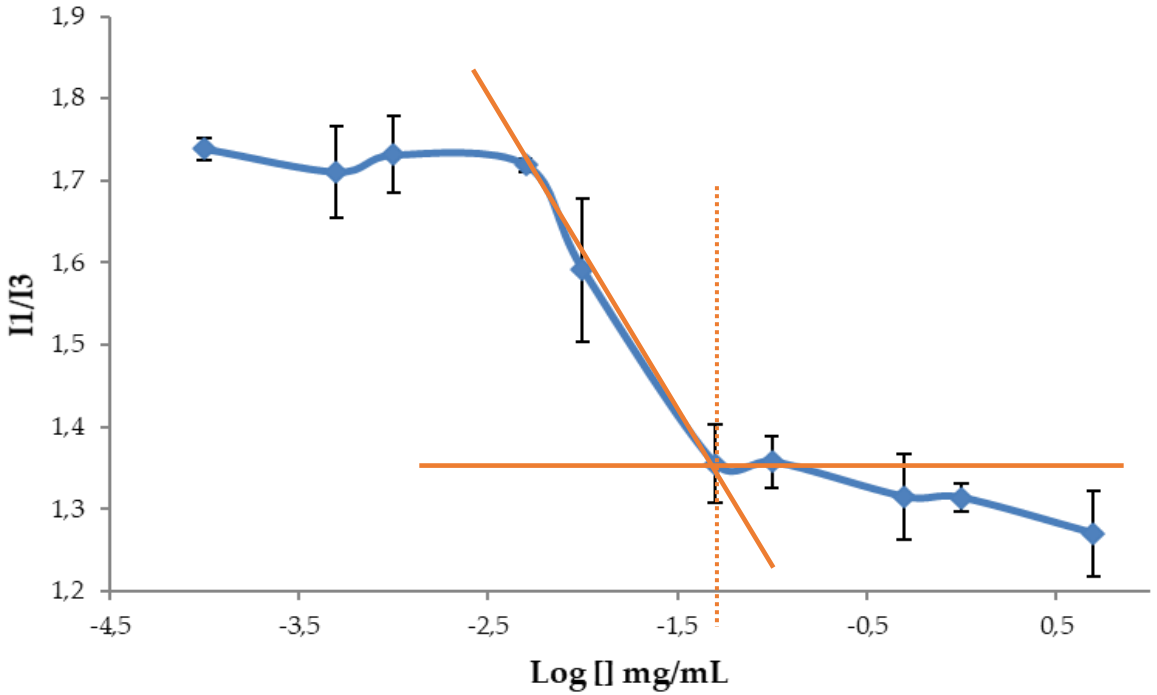
Results

Physicochemical characterization

Critical Micellar Concentration (CMC)

(Pyrene fluorescent probe)



CMC was around 50 µg/mL.

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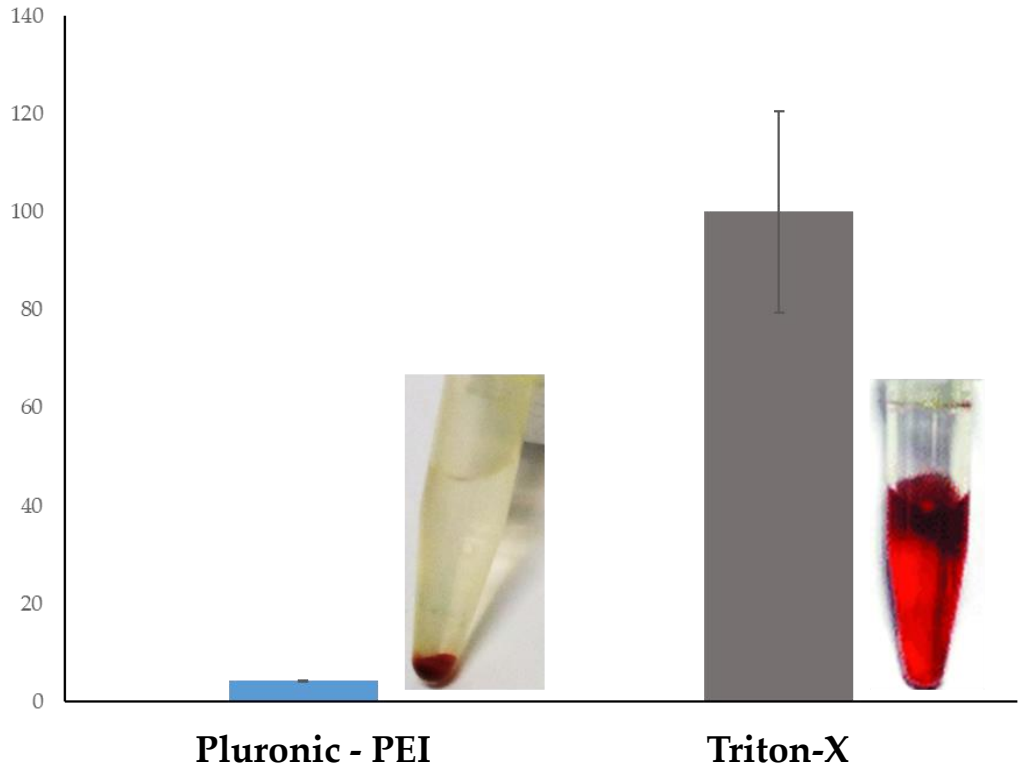
Results

In vitro biocompatibility

Hemolysis test



Hemolysis ratio of a 5 mg/mL nanomicellar solution was less than 5%.

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Conclusions

- A novel Pluronic L121-PEI was successfully synthesized which can 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.

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Acknowledgments

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Thank you very much for your attention!

OBRIGADA!

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