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Formulation of biocompatible nanoemulsions to stimulate the culture of Circulating Tumour Cells (CTCs) in vitro

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Introduction

The study of Circulating Tumour Cells (CTCs) have gained attention during the last years due to their applicability in Liquid Biopsy related with the detection and progression of cancer metastasis. Some of the potential clinical applications for CTC analysis include early cancer detection, disease staging, monitoring for recurrence, prognostication and to aid in the selection of therapy. However the main inconvenience related to the study of CTCs is their low concentration in blood (1 CTCs per ?10^7 white blood cells (WBCs)) and the difficulty to culture them, for further *in vitro* assays.

Nanotechnology is a tool that offer great advantages in nanomedicine, particularly nanoemulsions which have the ability to interact with the cellular machinery by entering the cells through different cellular pathways, as opposed to many common small drugs or free substances that simply diffuse according to their solubility. Therefore, we propose an interesting and novelty approach to improve proliferation of CTCs, based in the formulation of nanoemulsions built up from phospholipids or fatty acids involved in the metabolic activity of metastatic cancer cells. These nanoemulsions will give the opportunity to delivery intracellularly, bioactive phospholipids which can interfere in the metabolic cell pathways to activate their metabolism abandoning their quiescent state.

Objectives

The main objective is the formulation of nanoemulsions built up from phospholipids with the ability to potentiate the proliferation of CTCs, previously isolated from breast and prostate metastatic cancer patients, with the aim to culture them *in vitro*. Moreover, these formulations can be used in other cancer cell lines too, as a tool to increase their proliferation in culture.

Methods

The nanoemulsions were prepared by the previously reported low-energy emulsification oil in water method and were physically characterized. Mean average size, polidispersion index (PI) and Zeta potential (ZP) were determined by ZetaSizer. The activity of the nanoemulsions was evaluated in vitro by cell viability assays (alamarBlue®): Moreover uptake of the nanoemulsion by breast cancer cell lines was proved using confocal laser scanning microscopy.

Results

Nanoemulsions using several phospholipids and fatty acids were successfully formulated in a wide range of sizes. Their chemico-physical characterization was done showing adequate colloidal stability in different media and with time. The cell viability assays to test the activity of the nanoemulsions related to their pro-proliferative activity showed a concentration and cell line dependence. Cellular uptake of the nanoemulsions was confirmed by confocal microscopy images.

Conclusions

We believe that our nanoemulsions are a novelty tool that will help in the generation of stable cell lines of CTCs in the future and in other areas of research related with proliferation as for example, tissue engineering.

1. We have obtained coloidally stable nanoemulsions formulated with different components. Adicionally, we were able to modify their sizes openning their applicability for different areas of nanomedicine.
2. It was proved that nanoemulsions increased the cell viability of breast cancer lines, although this behavior resulted to be cell line and concentration dependent.

3. Preliminary experiments with CTCs showed that nanoemulsions are not cytotoxic, but no conclusion can be drawn about the increase in CTC proliferation from the recorded data till the date.