O7-5	**Autologous magnetic biogenic vesicles for multimodal anti-tumoral therapy**

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INTRODUCTION AND OBJECTIVES

Cells are subjected to a variety of stimulation leading to the release of submicron fragments from the plasma membrane, usually termed cell microparticles or microvesicles, that participate in the intercellular communication (Piccin 2007). Loading autologous vesicles with magnetic nanoparticles (Luciani 2010) could result in a hybrid multifunctional biogenic vector for drug targeting. On the one hand, the biological origin of cell vesicles could facilitate their circulation *in vivo* and interaction with cells since their membrane hallmarks could favor cell recognition and fusion to deliver their cargo intracellularly. On the other hand, their magnetic content would enable magnetic resonance imaging and targeting by magnetic guidance while providing therapeutic potential through magnetically induced hyperthermia.

In this study, we aimed to develop such biogenic vesicles from macrophages previously loaded with both magnetic nanoparticles and a photosensitizer drug, *meso*-tetra(3-hydroxyphenyl)chlorin, *m*-THPC. Vesicle uptake by cancer cells was addressed.

MATERIALS AND METHODS

In order to produce magnetic vesicles loaded with a photosensitizer, the first step consisted in promoting the uptake of magnetic nanoparticles (6-nm maghemite particles stabilized by citrate ions) and the drug. As a second step, the release of magnetic photosensitizer-loaded vesicles was triggered by culturing such cells in serum-deprived medium.
Concerning the first step, we investigated the influence of magnetic nanoparticles (5mM iron) on drug uptake by macrophages derived from THP1 cell line. The uptake kinetics and the effect of m-THPC concentration (0.2, 1, 5, 10 µM) in this process was studied. In order to analyse photosensitizer uptake, treated cells were lysed in a detergent solution (Triton 100 X) and photosenstizer concentration was measured using a fluorescence spectrometer (Fig. 1 and 2).

After drug/magnetic nanoparticle co-incubation or independent incubation, the macrophages underwent a 24-hour culture in serum-deprived medium to trigger vesicle release. The photosensitizer concentration was determined in the obtained conditioned medium by spectrometry.

A micromagnetophoresis experiment was performed to investigate the magnetic mobility of the obtained vesicles. In this experimental set-up, we analysed the magnetophoretic velocity of the individual single vesicles moving towards a 50-µm-diameter nickel rod submitted to a 0.1 T uniform magnetic field from a rectangular magnet. Not only bright field images were acquired but also fluorescence ones in order to determine the presence of the fluorescent drug m-THPC inside the vesicles (Fig. 3).

As a next step, we investigated the ability of photosensitizer-loaded magnetic vesicles to transfer their cargo into tumor cells. In order to confirm that the transfer of photosensitizer was a vesicle-mediated process, macrophage membrane was stained by PKH67 dye before vesiculation in serum-deprived medium. The obtained vesicles were incubated with PC3 cancer cells during 24 hours in the presence of a magnetic field gradient to estimate *in vitro* a magnetic targeting effect.

RESULTS AND DISCUSSION

Magnetic nanoparticles enhance photosensitizer uptake by macrophages
An increased intracellular concentration of m-THPC was observed when incubation with macrophages was carried out in the presence of magnetic nanoparticles (5mM and 2-hour incubation) (Fig. 1). As magnetic nanoparticles are internalized by endocytosis, m-THPC may take advantage of this pathway to further enter the cell. The uptake kinetics in the presence of magnetic nanoparticles is presented in Fig. 2. A quite linear time-dependent uptake was observed and saturation was not achieved even at 4 hours of incubation.

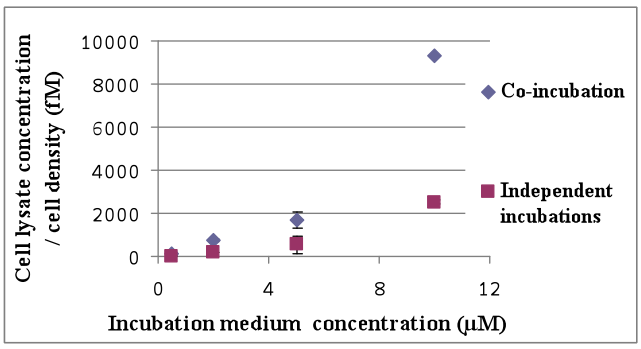

Figure 1: m-THPC internalisation by macrophages as a function of the photosensitizer concentration in the incubation medium and the presence of magnetic nanoparticles (5mM): co-incubation or independent incubations (2h).

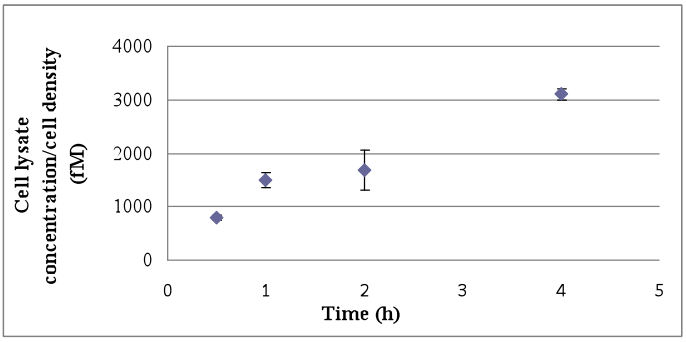

Figure 2: Kinetics of m-THPC internalisation (incubation medium at 5µM m-THPC, co-incubation with magnetic nanoparticles at 5mM).

Biogenic vesicles released by macrophages were both magnetic and photosensitizer-loaded

After co-incubation or independent incubation, the macrophages underwent a 24-hour culture in serum-deprived medium for vesicle release. Photosensitizer quantification in the conditioned medium obtained from macrophages treated with m-THPC at 5 µM for 2 hours in co-incubation with magnetic nanoparticles at 5mM or after independent incubations was 120 ± 9.7 and 24 ± 1.2 nM, respectively. As expected, intra-vesicle m-THPC concentration was higher when vesicles were obtained from cells in which drug and magnetic nanoparticles were co-incubated.

Concerning the micromagnetophoresis experiment (Fig. 3), the observed vesicles presented both magnetophoretic mobility and red fluorescence emission due to photosensitizer. This result indicates that drug-loaded magnetic vesicles were indeed produced.

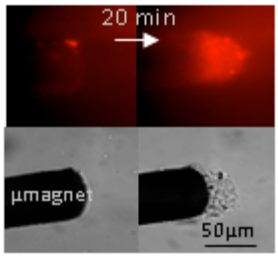

Figure 3: Micromagnetophoresis experiment: vesicles released by macrophages are attracted by a micromagnet and emit red fluorescence from photosensitizer. Top: Fluorescence images. Bottom: Bright field images.

Magnetic biogenic vesicles loaded with a photosensitizer are uptaken by cancer cells

Confocal microscopy was carried out in order to detect photosensitizer transfer from vesicles to PC3 cells by red fluorescence emission. According to the obtained images, red fluorescence was scarcely observed when PC3 cells underwent incubation with photosensitizer-free magnetic vesicles (Fig. 4a). However, red fluorescence was markedly emitted by PC3 cells after incubation with photosensitizer-loaded magnetic vesicles (Fig. 4b).Green fluorescence emission from vesicle membrane was observed on PC3 cells after incubation with photosensitizer-free vesicles (Fig. 4c). Only after incubation with photosensitizer-loaded vesicles**,** a co-localization was observed for green fluorescence from vesicle membrane and red fluorescence from photosensitizer (Fig. 4d). This indicates that m-THPC internalization by cancer cells did not take place by means of free photosensitizer in solution; instead, this process involved m-THPC-loaded vesicles. Such uptake could be spatially modulated by a magnetic field gradient. As observed on Fig. 4e, red-fluorescence markedly increased towards the magnet vicinity.

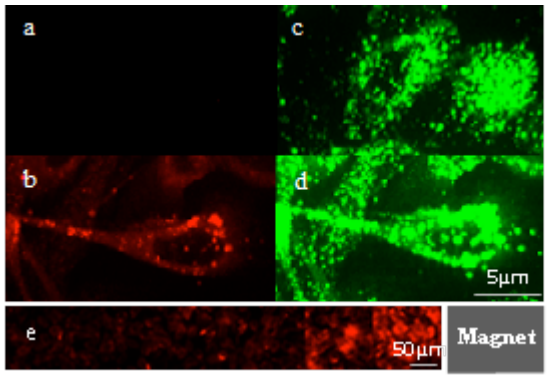

Figure 4: Red fluorescence emission by PC3 cancer cells after incubation with photosensitizer-free (a) and photosensitizer-loaded magnetic vesicles (b). Green fluorescence emission from vesicle membrane observed on PC3 cells after photosensitizer-free (c) and photosensitizer-loaded magnetic vesicles (d) uptake. Effect of the magnetic field gradient on the uptake of photosensitizer-loaded magnetic vesicles (e).

CONCLUSIONS

Photosensitizer-loaded magnetic vesicles derived from macrophages were successfully produced. Such new biogenic vector was able to transfer its cargo to tumor cells presenting then a therapeutic potential. Additionally, its magnetic properties may enable magnetic resonance imaging and targeting while magnetically induced hyperthermia may enhance the therapeutic action of the loaded drug.

ACKNOWLEDGEMENT

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REFERENCES

- Piccin et al (2007). *Circulating micronanoparticles: pathophysiology and clinical implications*. Blood Reviews 21, 157–171.
- Luciani et al (2010). *The role of cell-released microvesicles in the intercellular transfer of magnetic nanoparticles in the monocyte/macrophage system*. Biomaterials 27, 7061-7069.