Hyperthermic superparamagnetic nanoparticles modulate adipocyte metabolism

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Background

Adipocytes are the principal cellular component in adipose tissue and their excessive hyperplasia or hypertrophy is actively involved in regulating physiologic and pathologic processes such as inflammation, cardiovascular disease, obesity and tumour. The main depot of energy in adipocytes is represented by lipid droplets, intracellular organelles that play fundamental roles in regulation of metabolic processes. An accumulation of such droplets could be a potential biomarker of disease caused by metabolic dysregulation. Recent studies have demonstrated that heat shock is associated with alteration in energy metabolism: the aim of this study is to modulate the energy metabolism of the adipocytes via controlled administration of thermal energy to reduce the number of lipid droplets. We have investigated the effect of controlled heating of adipocytes using an alternating magnetic field (AMF) on samples loaded with superparamagnetic nanoparticles (MNP) as heating agent.

Methods

Superparamagnetic nanoparticles (iron oxide) (MNP) were synthesized by solvothermal decomposition in high boiling solvent as described in Park J et al. [1]. MNP were coated by an amphiphilic polymer (poly(isobutylene-alt-1-tetradecene-maleic anhydride)) (PMA). The resulting PMA-coated nanoparticles (PMNP) were dispersible in aqueous medium.

The hyperthermic efficiency of PMNPs was assessed by measuring the Specific Absorption Rate (SAR) [2] applying an AMF of 521 KHz and 25 kAm^{-1}(Magneterm 1.5). The temperature variation of the PMNPs suspension was measured with a thermometer equipped with optical fiber probes and recorded continuously during the application of AMF.

3T3-L1 preadipocytes were induced to adipocytes with an adipogenic medium according to Zebisch K et al. [3]. 7-days post-induction cells are considered as mature adipocytes [3].

PMNPs were conjugated with Fluorescein isothiocyanate (FITC) to assess their internalization and uptake in adipocytes by confocal microscopy and flow cytometry.

The PMNPs internalization was also evaluated with Transmission electron microscopy (TEM). Cells were fixed with glutaraldehyde and paraformaldehyde, post fixed with OsO_4 and embedded in Epon resin.

Viability of adipocytes loaded with PMNPs was evaluated by MTT test and by Trypan blue exclusion test at different times and concentrations. These preliminary results were used to determine the condition to maximize the PMNPs uptake (incubation time: 24h, PMNPs concentration 50 µg/mL).

The cells then received a single hyperthermic treatment by exposing the samples to AMF (f = 521 KHz H_0= 25 kAm^{-1}) for 20 min.

To determine the lipid accumulation, the cytoplasmic triglycerides forming lipid droplets were detected by staining with Oil Red O solution. The stained cells were first photographed using a light microscope followed by semi-automatic quantifications of droplets using an image processing software (ImageJ).

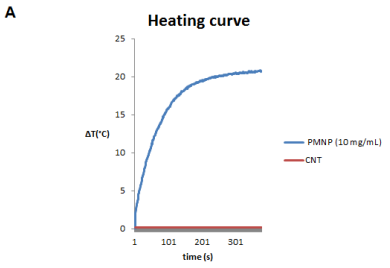
The Oil Red O retained in the cells were then extracted with isopropanol and spectrophotometrically quantified. The absorbance was determined at 490 nm.

To analyze the expression of genes involved in the adipogenesis (adipsin; leptin; adiponectin; peroxisome proliferator-activated receptor γ, PPARγ; enhancer binding protein alfa, C/EBPA; fatty acid binding protein 4, AP2; perilipin 1, PLIN1), in the lipolysis (adipose triglyceride lipase, ATGL; monoglyceride lipase; MGLL) and in cell growth and apoptosis (p53, p21, cyclin d1, puma) total RNA was isolated from cultured cells. The total RNA extracted was then subjected to quantitative real time reverse transcription.

Statistical differences between treatments were evaluated using ANOVA analysis.

Results and discussion

SAR obtained from the heating curve of a PMNP sample with an iron concentration of 10 mg/mL was 131 W/g, as reported in Fig.1.



B

Mass [g]					Specific heat [J/g*K]		Slope (dT/dt)	SAR
Sample (g)	Fe [% w/w]	Solvent (g)	Particles (g)	Fe (g)	water	Particles	[K/s]	[W/g]
1,01	1	0,996	1,40E-002	1,01E-02	4,18	0,67	0,317	131,1

Figure 1 Temperature variation as a function of AMF exposure time measured on a sample of PMNP containing iron at a concentration of 10 mg/mL (A). SAR values as W/g of iron (B).

The MTT assay showed a not remarkable PMNPs cytotoxicity towards adipocytes as revealed by the high (>80%) viability of the cells after incubation (Figure 2).

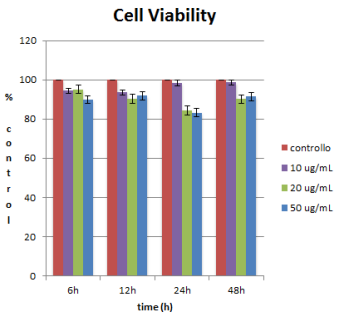


Figure 2 MTT Assay. Cells were treated with three different doses and three different concentrations of PMNP as indicated.

Confocal microscopy images associated with flow cytometry proved that PMNP were uptaken in a time and dose-dependent manner (Figure 4).

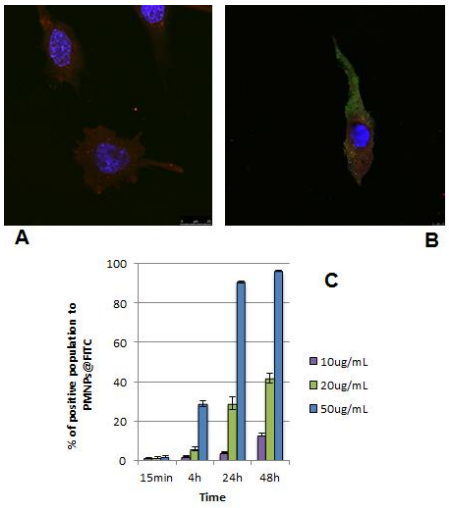


Figure 3 FITC-PMNPs internalization in adipocytes. Confocal microscopy images showed adipocytes incubated with 24h of 50 µg/mL (B) compared to control (A). FACS analysis (C) showed the percentage of adipocytes positive to FITC-PMNPs.

TEM analysis revealed a good PMNP uptake and an interesting distribution of free ranging nanoparticles in close proximity to lipid droplets.

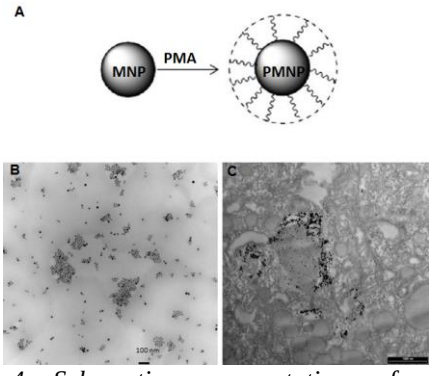


Figure 4 Schematic representation of magnetic nanoparticles (A). TEM image of PMNPs (B) and PMNPs uptake in adipocyte (C).

A sensible reduction of lipid accumulation was evaluated in cells treated with nanoparticles and exposed to AMF compared to control.

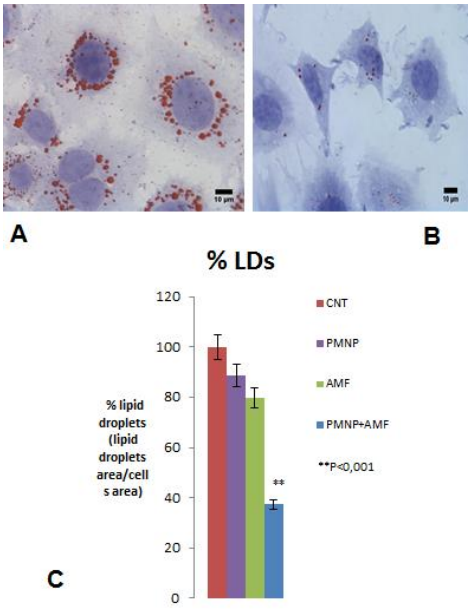


Figure 5 Adipocytes treated with hyperthermia (B) compared to untreated ones (A). Lipid droplets were stained with Oil Red O. Graphic representation of the lipid droplets reduction (C) after PMNP+AMF treatment.

Conclusion

We suggest the modulation of adipocytes metabolism in a non-invasive and innovative way based on the hyperthermic properties of PMNPs.

References

[1] Park J et al. Nat Mat, 3, 891-895, 2004.
[2] Xu R et al. IEEE Transactions on Magnetics, 3, 1078-1085, 2007
[3] Zebisch K et al. Analytical Biochemistry, 425, 88-90, 2012

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