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A catalase-magnetic switch for cell proliferation

Sofia Municoy, Irene L. Ibañez, Hebe Durán and Martín G. Bellino*

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The advance of novel biotechnology requires the development of versatile control systems for cell behavior. Towards the vision of cell growth control, we report a magnetic switching in cell proliferation by a catalase-nanomagnetite complex. The system synergically exploits both the ability of catalase to scavenge cell-generated hydrogen peroxide, a messenger involved in cell cycle regulation, and magnetite manipulation to modulate cell proliferation between arrest and growth using magnetic fields. This ON/OFF switch methodology could constitute the basis of new classes of diagnostic and therapeutic strategies as well as novel integrated responsive biomaterials.

Introduction

Organisms have evolved a wide variety of mechanisms to control cell growth which are crucial to the metabolism and reproduction of living systems. Scientists are currently focused on finding ways to mimic cellular regulatory mechanisms in order to develop new methodologies with potential diagnostic and therapeutic applications.[1] The ability to regulate cell proliferation in response to environmental conditions (e.g. substrate levels, exposure to growth factors and hormones) is vital to many metabolic functions.[2] Controlling signaling mediators is a key feature to actuate on cell behavior. Reactive oxygen species (ROS) are generated as by-products of cell aerobic metabolism. Although hydrogen peroxide (H_2O_2), a non-free radical ROS, is commonly known for its cytotoxic effect, at physiological levels it plays a role as an important messenger that regulates diverse biological processes.[3-4] In this sense, different regulatory proteins involved in signal transduction pathways can be oxidized by H_2O_2, leading to the activation or deactivation of these proteins, e.g. decreased levels of intracellular H_2O_2 culminate in the arrest of cell proliferation.[5-8] Catalase (CAT), an enzyme highly active and specific in decomposing H_2O_2, has been therefore used to inhibit cell growth.[5-8] Versatile methods to switch cell growth represent an evolution in biotechnological strategies, presenting a substantial scientific and engineering challenge for advanced biomaterials and biomedicine. Among addressable systems, magnetic nanoparticles (MNPs) stand out, because they can incorporate functional elements and provide magnetic control capabilities.[9-13] Magnetic fields are promising for remote stimulation because they can penetrate deeply into biological tissues with negligible attenuation.[14]

Taking advantage of these combined features, here we report a magnetic-field-assisted switching in cell proliferation by CAT-MNP nanostructured complex to turn on and off the proliferation of a human cell line. Actuation was achieved by remote magnetic switching to spatially put together the complex and cells for growth inhibition (OFF state) or separate them for proliferation (ON state). We show that the combination of enzymatic catalysis to block cell messengers with magnetic nanoparticles lead to a versatile route to control cell behaviors.

Results and discussion

Development and characterization of CAT-MNP complex

For preparation of the CAT-MNP nanostructured complex, CAT was conjugated to iron oxide nanoparticles (nanocrystalline magnetite, Fe_3O_4 with an average size of 10 nm; MNP, leading to a narrow nanocluster size distribution in aqueous solution of ca. 35 nm. See Supplementary Information, Fig. S1) through glutaraldehyde crosslinking of enzymes[15] in presence of MNPs.

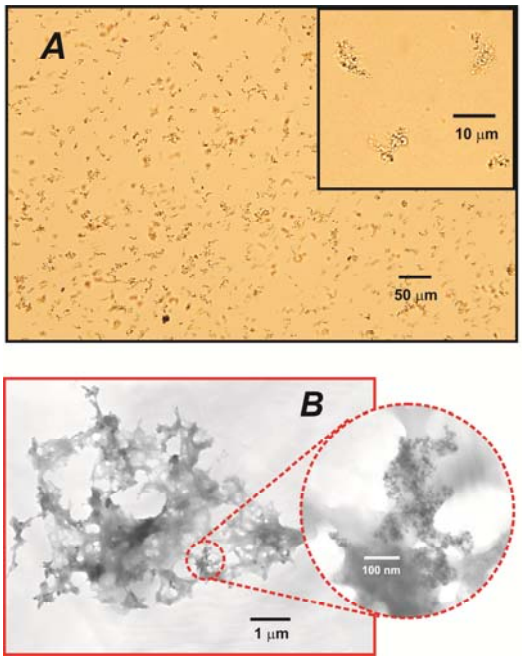

Fig. 1 Characterization of CAT-MNP complexes. Representative light microscopy (A) and TEM (B) images of CAT-MNP complexes. The blow-up of the TEM image shows the presence of entrapped magnetite nanoparticles.

The resulting material was a nanostructured network of fused enzyme micro-particles where MNPs clusters were physically entrapped during the cross-linking process. Further, in view of the magnetic properties of the nanoparticles, the resulting complexes can be easily separated from the reaction mixture by the application of a magnetic field, avoiding centrifugation or filtration. After magnetic separation, high catalase activity was

detected in the magnetic nanocomplex. The estimated capacity for enzyme loading in the CAT-MNP complex was 7 mg enzyme/mg magnetite. Moreover, nanostructured complexes have shown to be reusable, with no performance decrease, and substantially more active than the free enzyme at extreme pHs and temperatures, indicating that the nanocomplexes had a significantly enhanced stability (See SI Fig. S2). The CAT-MNP complexes were characterized by optical and electron microscopy to determine their morphology and size distribution as shown in Figure 1. This analysis revealed a matrix of fused enzyme entrapping MNPs with an average size of 10 µm. The entrapped MNPs appeared as aggregates with sizes of 0.2–0.5 µm, attributable to nanocluster agglomeration during the cross-linking process.

CAT-MNP complex to control the intracellular levels of reactive oxygen species

We evaluated the ability of the CAT-MNP to modulate cell oxidation state by determining the relative level of ROS in the OFF state (in presence of CAT-MNP) compared to the ON state (CAT-MNP removed by a magnetic field). Human A375 melanoma cells were incubated with CAT-MNP complexes.

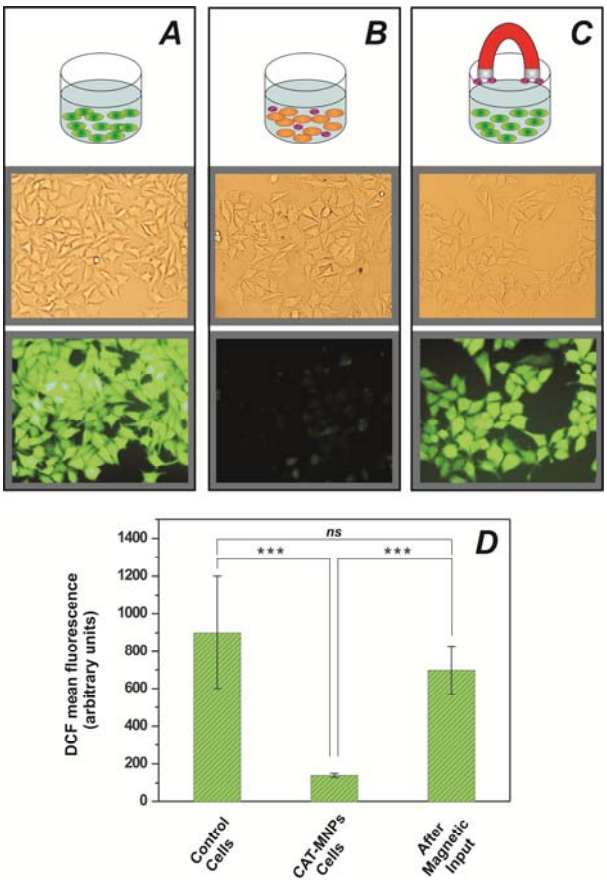

Fig. 2 Magnetic control of intracellular ROS levels of A375 cells, determined by DCFH-DA assay. Representative images of DCF fluorescence (bottom) and light microscopy (top), photomicrographs were taken with a 100 X magnification. (A: non-treated control cells, B: cells incubated with CAT-MNPs complexes, C: cells after removing CAT-MNPs complexes by a magnetic field). The schemes illustrate the different conditions evaluated. (D) Quantification of DCF mean fluorescence. Data are expressed as mean ± SD. ***p<0.001, ns: non-significant.

To gain evidence of the capability of CAT-MNP to remove ROS and in particular H_2O_2 of cell medium, intracellular ROS production was examined by dychlorofluorescein diacetate (DCFH-DA) assay and observed through fluorescence microscopy.[6] In Figure 2, we present a photographic series which illustrates the ability of the methodology to switch cell oxidation state. The presence of CAT-MNP (switch OFF) in the cell medium for 1 h decreased intracellular ROS levels, revealed by the low levels of oxidized DCF (low green fluorescence). Thus, considering that H_2O_2 can diffuse across membranes,[4] the addition of CAT-MNP complexes to the culture medium produced a decrease in the intracellular level of H_2O_2 from outside to inside the cell, reaching a lower steady state concentration. On the other hand, cells exhibiting a strong green fluorescence signal were observed after the removal of the complexes by the application of a magnetic field (Switch ON), associated with higher levels of ROS, comparable to control cells without treatment. Since endogenous H_2O_2 is involved in signal transduction pathways implicated in cell proliferation,[5-8,16] this magnetic switch can be used to actuate on the cell cycle in order to regulate cell growth.

Magnetic cell proliferation switch by CAT-MNP complex

We then examined the ability of the method to control cell proliferation. For switching OFF proliferation, A375 cells were incubated with CAT-MNP complexes, which were observed both evenly distributed and dispersed over time. Cell proliferation was evaluated by the MTT assay.[17] As shown in Figure 3A, a significant decrease of cell proliferation rate was observed in the presence of CAT-MNP complexes as compared to non-treated cells attributable to removal of endogenous H_2O_2, while cell proliferation was not affected by MNPs and heat-inactivated catalase. CAT-MNP complexes reached similar levels of cell growth inhibition when compared to free catalase and CAT-complex without MNPs (See SI, Fig S3). These findings demonstrate that inhibition of cell proliferation is mediated by catalase activity in the magneto-enzyme complex. Results showed in Figure 3B demonstrate the ability to magnetically activate cell proliferation (switch ON). Quantitative analysis of cell proliferation showed that cells incubated with CAT-MNPs exhibited a recovering of cell growth after exposure to a magnetic field (switch ON) whereas cells without the magnetic activation remained inhibited (switch OFF). This magnetic activation of cell proliferation did not occur when cells were treated with catalase complexes in the absence of MNPs, reinforcing the hypothesis that all the three components of the system (magnetic field, catalase and MNP) are necessary to elicit the ON/OFF switch on cell proliferation.

The efficacy of reversing the inhibition of proliferation was higher for the magnetic switch than for the removal of the complexes by replacing the medium with fresh one (Figure 3B). This could be explained by the maintenance of the conditioned culture medium (containing growth factors released by the cells)[18] when CAT-MNP was removed by using the magnetically activated system. This comparison illustrates an advantage of being magnetically switched ON to activate cell proliferation in a remotely and non-invasively way. As shown in Figure 3C the remote magnetic activation was able to actuate the on/off cell growth, with the ON state producing on average 35-fold higher cell proliferation than the OFF state after 48 h. Furthermore, after 48 h of magnetic activation, non-significant differences in cell

proliferation rates were found as compared to non-treated cells.

Ageing effects of the CAT-MNP complexes should be carefully studied, in order to extract an activity lifetime window necessary for practical applications. So far, preliminary results showed that CAT-MNPs could maintain a significant arrest of cell proliferation up to 10 days incubation, thus suggesting that long-term inhibition of cell growth can be reached.

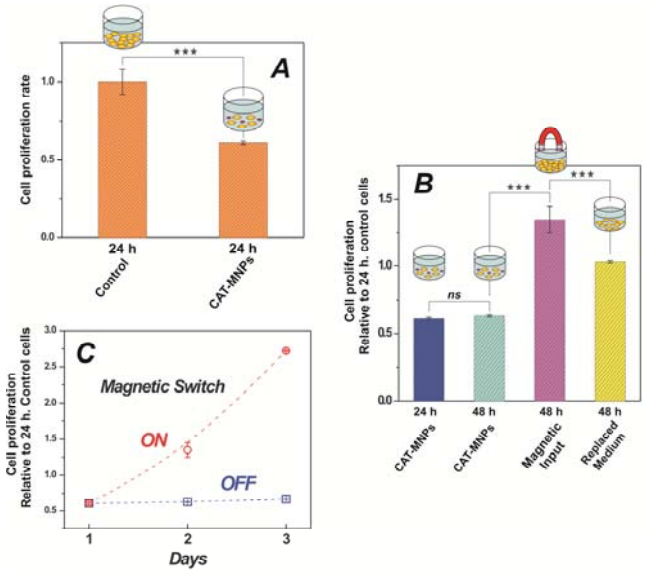

Fig. 3 Magnetic switch in melanoma cells proliferation, evaluated by the MTT assay. (A) Proliferation rate of A375 cells treated with CAT-MNP complexes for 24 h, relative to non-treated control cells. (B) Comparison of proliferation of A375 cells treated with CAT-MNP complexes for 24 h or 48 h and proliferation of A375 cells treated with CAT-MNP complexes for 24 h followed by removal of these complexes by magnetic field exposure for 30 min or by replacing the medium with fresh one and leaving these cells grown for another 24 h. The schemes on top illustrate the different conditions evaluated. Data are expressed as mean ± SD. ***p<0.001, ns: non-significant. (C) A375 cell proliferation vs time under treatment with CAT-MNPs complexes (□) or following their removal by a magnetic field after 24 hours of treatment (○).

Conclusions

In summary, we have designed and demonstrated a novel and versatile methodology to turn cell proliferation on and off by means of magnetic control in a temporarily controlled manner. This switching cell growth system is a proof of concept of the potential use of enzyme-magnetite complexes to scavenge mediators of signal pathways to switch cell responses by the application of a magnetic field. Our approach could activate cells uniformly across a large volume, making it feasible for in vivo applications.

A platform based on catalase-magnetite complexes has been developed. Cell proliferation was turned OFF by catalytically removing cell-produced H_2O_2, a messenger implicated in cell proliferation. The magnetic switch turned into its ON mode when a magnetic field was applied to separate nanomagneto-catalase complexes from cells, promoting proliferation. Since control cell proliferation is one of the main bases in regenerative medicine,[19] this development would be of great importance for improving tissue engineering and integration of prosthesis and implants.

This system provides additional opportunities for controlling other cell behaviors, which have H_2O_2 mediators as key features, so dissimilar that span from immune regulation and vascular remodeling to stomatal closure.[20] Furthermore, the compatibility of this approach with the vast library of enzymes that are potentially available to head off cell-generated mediators may create new approaches to integrated systems for controlling cellular activities, such as differentiation, metabolism and apoptosis. This simple yet robust methodology has therefore the potential to open a new avenue for exploring novel diagnostic and therapeutic scenarios. Finally, considering that products of interest are synthesized by cells depending on different growth states,[21] a straightforward combination of this control system with cell-integrated materials to allow the design of novel classes of devices with magnetic-responsive behavior can be envisioned for a broad range of applications.

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Departamento de Micro y Nanotecnología, Comisión Nacional de Energía Atómica, Av. Gral Paz 1499 (B1650KNA), San Martín, Buenos Aires, Argentina.
E-mail: mbellino@cnea.gov.ar

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Table of Contents:

The combination of enzymes to tackle cell messengers with magnetite nanoparticles was exploited to control cell behavior by means of magnetic fields.