Advanced Nanomaterials for Remotely Controlled Therapies

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Enzyme prodrug therapy (EPT) represents a promising and versatile approach for the spatial and temporal control of therapeutic activation through the catalytic **conversion of inert prodrugs into active molecules by exogenous enzymes** [1]. One of its most relevant applications lies in the development of antitumor strategies for the *in-situ* generation of chemotherapeutic agents from non-toxic prodrugs, providing a means to enhance local drug efficacy while minimizing systemic toxicity. By enabling the localized formation of cytotoxic compounds from systemically safe prodrug doses, which are non-toxic and cannot be converted by endogenous enzymes, EPT has the **potential to overcome the long-standing trade-off between chemo-therapeutic efficacy and systemic toxicity** that limits conventional chemotherapy [1].

Despite its conceptual promise, EPT translation has been limited by enzyme instability, rapid degradation, immune clearance, and insufficient spatial control of prodrug activation. Most reported EPT systems rely on **mesophilic enzymes**, whose maximum catalytic efficiency for prodrug bioconversion occurs at physiological temperature (37 °C). Although highly substrate-specific, **these enzymes can** still **trigger off-target prodrug activation** outside the intended site, compromising therapeutic precision and safety [1].

To address the limitations of conventional EPT, **we propose a novel strategy based on thermo-responsive enzyme nanohybrids** capable of remote and localized activation. Unlike mesophilic enzymes, which are highly active at 37 °C, **thermophilic therapeutic enzymes** exhibit low prodrug-converting activity at physiological temperature and reach their maximum catalytic potential only at elevated temperatures (>50 °C). To enable controlled activation of their high-temperature catalytic potential, we have developed **thermo-responsive nanohybrids** that co-integrate the enzyme with magnetic nanoparticles (MNPs) within a porous silica matrix, forming a fully **synergistic system**. Each component plays a critical role (Figure 1): i) thermophilic enzyme, provides high catalytic efficiency, but only when its local environment reaches the optimal temperature; ii) magnetic nanoparticles (MNPs), act as local nanoheaters and upon absorption of energy from an alternating magnetic field (AMF), dissipate this energy as **localized heat**, selectively warming the enzyme's microenvironment without affecting the global tissue temperature. iii) porous silica matrix, ensures spatial confinement, thermal insulation, prevents unwanted heat diffusion, stabilizes the enzyme against proteolytic degradation in biological media, and reduces immune recognition and clearance, enhancing *in vivo* persistence.

Upon AMF exposure, the synergistic action of these components triggers efficient prodrug-to-drug conversion. Importantly, this **localized magnetic heating** does not directly induce cell death; rather, it **activates the enzyme**, which generates the cytotoxic chemotherapeutic in situ, **enabling remote, spatially and temporally controlled therapy** with minimal off-target effects (Figure 2).

As a proof of concept, this approach was tested in **pancreatic ductal adenocarcinoma (PDAC)**, a malignancy with <11 % five-year survival and poor response to current chemotherapies due to dense stroma and limited vascularization [2]. Using horseradish peroxidase (HRP) as the therapeutic enzyme and the non-toxic prodrug indole-3-acetic acid (3-IAA), the nanohybrids allowed AMF-controlled generation of reactive oxygen species (ROS), inducing selective tumor cell death. The nanohybrids preserved enzymatic activity after AMF application, exhibited up to 150-fold enhanced stability against heat and proteolysis, and doubled catalytic efficiency upon AMF activation. In MIA PaCa-2 **pancreatic cancer cell cultures (2D)**, remotely activated EPT achieved >95 % cytotoxicity, while **xenograft mouse models** exhibited complete tumor growth suppression exclusively under AMF exposure. Remarkably, 85% of the nanohybrids were retained in the tumor after 1 month, with no weight loss observed in mice, indicating good treatment tolerability [3].

Beyond PDAC, **this thermo-responsive EPT concept is modular and adaptable to diverse tumors**, providing a generalizable platform for localized, low-toxicity, and remotely controllable enzyme-based therapies capable of addressing critical unmet clinical needs.

References

[1] Beatriz Torres-Herrero, Ilaria Armenia, Cecilia Ortiz, Jesús Martinez de la Fuente, Lorena Betancor, Valeria Grazú, J. Control. Release, 372 (2024) 619-647.
[2] Hordur Mar Kolbeinsson, Sreenivasa Chandana, G Paul Wright, Mathew Chung, J Invest Surg. 26(1) (2023) 2129884.
[3] Beatriz Torres-Herrero, Ilaria Armenia, Maria Alleva, Laura Asín, Sonali Correa, Cecilia Ortiz, Yilian Fernández-Afonso, Lucía Gutiérrez, Jesús M de la Fuente Lorena Betancor, Valeria Grazú, ACS Nano, 17(23) (2023) 12358-12373.

Figures

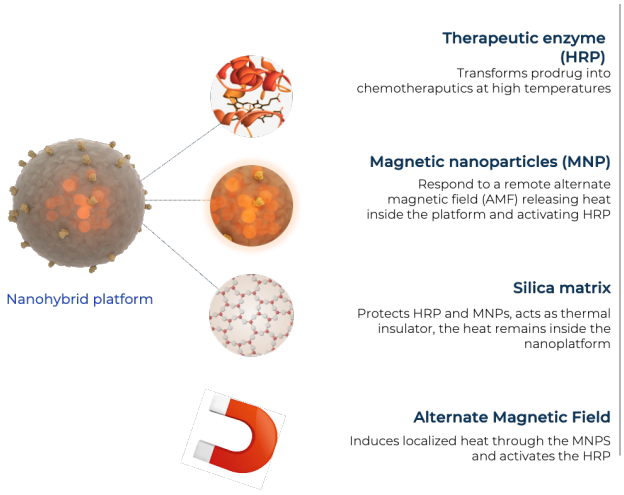

Figure 1. Illustration of the nanohybrid system, showing the main components and the remote stimuli that induce activation.

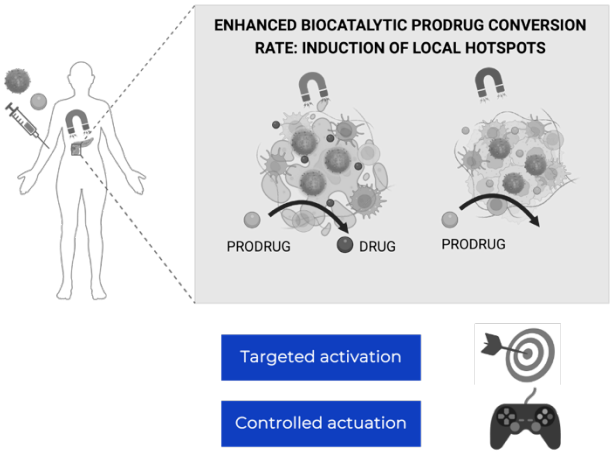

Figure 2. Schematic illustration of the proposed novel EPT strategy using thermo-responsive enzyme nanohybrids activated by AMF.