Prodrug activation by biocatalytic nanoparticles: an emerging area in nanomedicine

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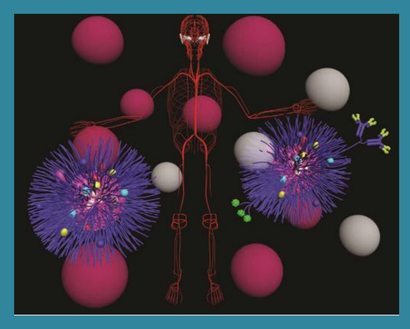


Introduction & State of the art

In the past years, nanomedicine has become an important tool to develop new and also more efficient cancer diagnostics and therapies. Selecting the precise cell targets and the appropriate nanocarriers is the principal aim of projects in nanomedicine.

Current Cancer Chemotherapies

The major drawback with current treatments is the prevalence of unrequired dose-limiting toxicity to non-cancerous tissues. Moreover, antitumor drugs often show low solubility and trend to be deactivated or be degraded in blood circulation.

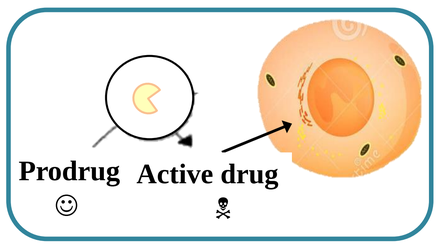
Prodrugs and direct enzyme-prodrug therapy (DEPT)

Drugs precursors do not show cytotoxic activity because they need to be activated by the exogenous enzyme. Different targeting strategies as a Antibody-directed, Gene-directed and Virus-directed enzyme prodrug therapy have improved the treatments but they have also shown some disadvantages: increasing mutation rate, low cell transformation and immunoreactivity are some of them.

NANOREACTORS

This strategy has to use biocompatible nanosystems (10-100nm) in order to transport the active enzyme to the tumor, where the catalytic reaction will be carried out. Low systemic toxicity, effective dose, increased enzyme stability and less immonoreativity can be accomplished with nanoreactors.

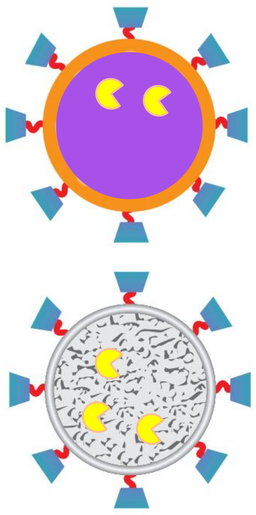
It is required that the nanoreactors show selective permeability and low aggregation capacity.



Types of nanoreactors

Polymers

They represent the most heterogeneous group. The encapsulation can take place via the simplest method (physisorption) and the enzyme usually can remain inside because of electrostatic interactions.

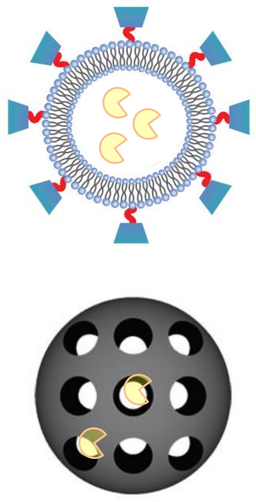


Metallic Inorganic Nanoparticles

They have an extensive range of characteristics suitable for vectorization: rich functionality, optical and magnetic properties (useful in diagnostic). Among them, gold nanoparticles (AuNPs) are the most common ones.

Enzymosomes

Based on lipids. They have amphipathic character and high degree of biocompatibility. The most studied are liposomes even though they are not the most used in enzyme delivery.



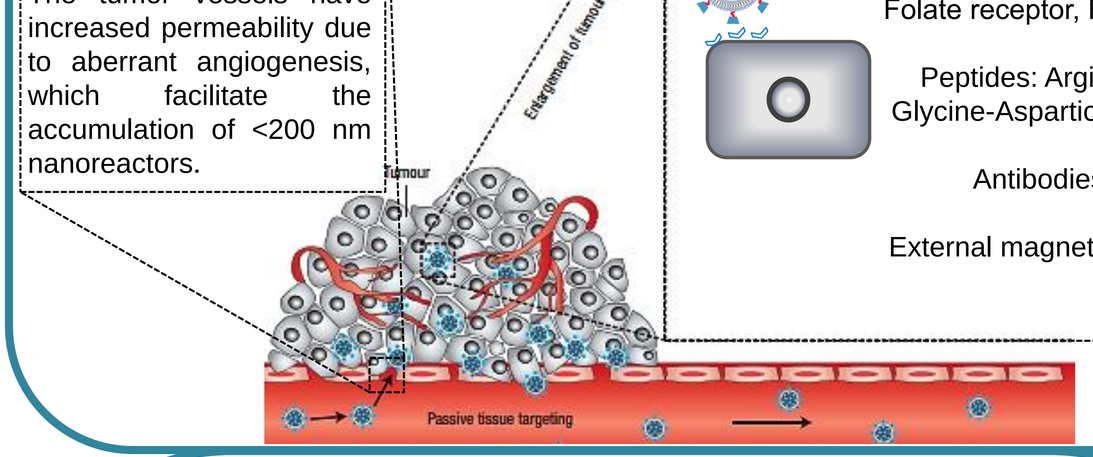
Mesoporous silica nanoparticles

They are promising candidates due to their unique properties, such as easy control of the pores diameter during the synthesis, high load capacity and their robustness. Furthermore, they show low cytotoxicity and are biodegradables.

Tumor Targeting

Passive tumor targeting (Enhance Permeation and Retention)

The tumor vessels have increased permeability due to aberrant angiogenesis, which facilitate the accumulation of <200 nm nanoreactors.



Active tumor targeting

Cell proliferation markers: Folate receptor, EGFR…

Peptides: Arginine-Glycine-Aspartic (RGD)

Antibodies

External magnetic fields

Some examples

- Mesoporous silica nanoparticles were selected as the material for the D-Amino acid oxidase (DAO) delivery system. This enzyme catalyze the formation of hydrogen peroxide with strong oxidability and cytotoxicity.

  The adsorption, activity and stability (Figure 1A) of DAO are demonstrated to depend mainly on the amino-functionalization surface.

  Significant cytotoxic effect has been observed when the cells are treated by the nanoreactor together with D-Alanine (Figure 1B).

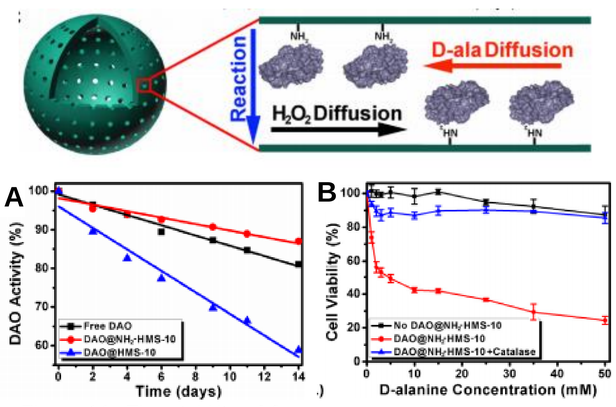


Figure 1A. The stability of free DAO and DAO encapsulated (immobilized or not).

Figure 1B. Cytotoxic effect of the nanoreactor.

- Studies with ABA triblock copolymer that encapsulate *Trypanosoma vivax* nucleoside hydrolase (TvNH) are another experimental approximation to the therapy with nanoreactors. This enzyme catalyzes the hydrolysis of nucleoside analogues (as some antitumor drugs). To allow the entry of the substrate, OmpFor or Tsx, membrane proteins, were added.

  The results show that the enzyme activity depends on the OmpFor/polymer ratio (Figure 2).

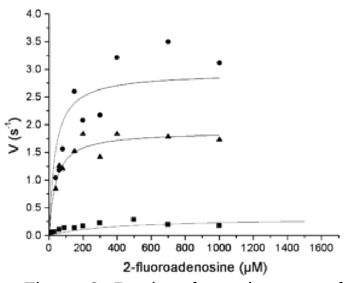


Figure 2. Product formation rate of nanoreactors permeabilized with different ratios of membrane protein

- Another, even more newly experiment used magnetic nanoparticles (~30-60 nm) to encapsulate E.Coli cells, previously transformed with a vector that overexpressed the enzyme cytosine deaminase under the action of a temperature inducible promoter.

  The expression of the enzyme induced by magnetic fields took place at temperatures of 42-43ºC causing cell death but not at physiological conditions (Figure 3).

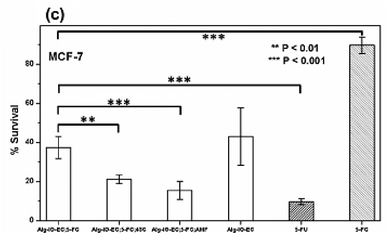
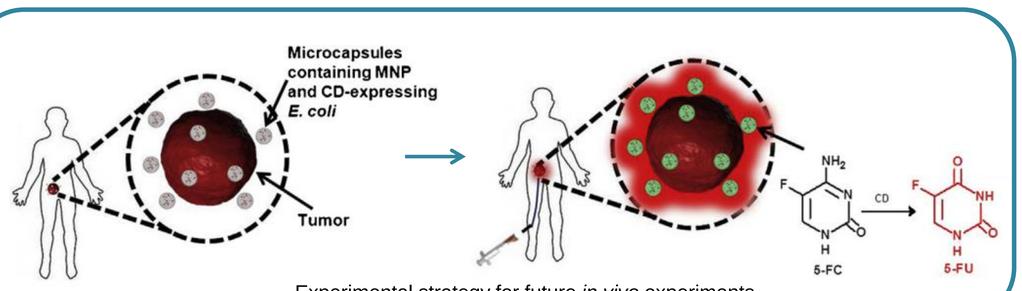
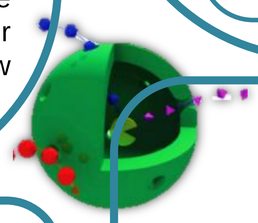


Figure 3. Tumor cell cytotoxicity in MCF-7, human breast cancer cell after the treatment.



Experimental strategy for future *in vivo* experiments



Conclusions

Nanotherapy has brought improvements and efficiency to the current chemotherapy: more enzyme stability, low immunoreactivity and less side effects are some examples of what it has provided. New approaches based in increasing the catalytic activity and the encapsulating efficiency will let nanotherapy position as the principal strategy for clinic studies .

One can predict that, in the future, we will be able to combine *in situ* nanoreactors with X-ray technics to optimise the anticancer treatment.

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