Human lactate dehydrogenase heterogeneous biocatalyst for efficient anticancer drug screening

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Summary

A worldwide study conducted by International Agency for Research on Cancer (IARC) in 2020 estimates 19.3 million new cancer cases and according to the IARC survey, they are expected to increase to 30.2 million in 2040. For this reason, Research continues to focus on new therapies and treatments for cancer. It has been proved that several cancer cells present a different metabolic pathway in comparison with healthy cells, during which glucose is consumed to produce energy by high-rate glycolysis followed by lactic acid fermentation. The enzyme lactate dehydrogenase (LDH), whose A isoform is overexpressed in cancer cells, has an important role in this altered metabolic pathway. LDH-A catalyzes the reduction of pyruvate to lactate, oxidizing the cofactor NADH to NAD^+ at the same time. For this reason, lactate dehydrogenase A (LDH-A) can be looked upon as a target of antitumoral drugs. Indeed, several studies demonstrate that the inhibition of LDH-A has positive effects in terms of reduction of tumor growth and proliferation. At present, the methods used to test the efficiency of LDH-A inhibitors include expensive and time-consuming analyses because, in the first screening phase of new inhibitory molecules, each compound must be tested on the enzyme that can not be recovered. The immobilization of enzymes on solid supports allows the reusing of the biocatalyst and enables its use in different types of reactors.

aims to explore the possibility of using an immobilized enzyme to evaluate the inhibitory effects of potential chemotherapeutic compounds. Inhibitors of lactate dehydrogenase will be examined as potential anticancer drugs due to the key role of this enzyme in cancer cells' metabolism. The proposed device is formulated as a

heterogeneous biocatalyst coupled to an amperometric sensing unit, and it is schematized in the **Figure** below.

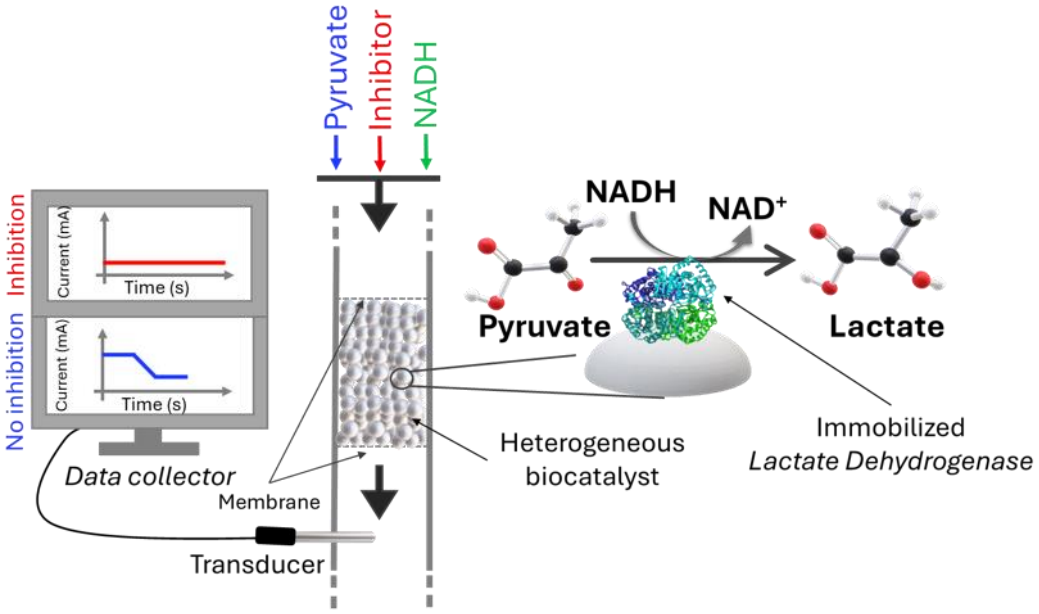

Figure. Simplified representation of the two-section amperometric sensor for screening Lactate Dehydrogenase inhibitors

Specifically, this research will focus on the following objectives:

- To achieve an active and durable lactate dehydrogenase heterogeneous biocatalyst by assessing two different covalent immobilization methods, varying the stabilizing agents and the immobilization conditions.
- To evaluate the impacts of varied physical and chemical properties of siliceous supports on the activity of immobilized lactate dehydrogenase.
- To evaluate if the immobilized enzyme can be used to estimate the efficacy of lactate dehydrogenase inhibitors.
- To select a suitable sensing system to couple with the heterogeneous biocatalyst to monitor the lactate dehydrogenase activity.

The results obtained will provide valuable insights that will guide the design and development of devices useful for screening new pharmaceutical compounds formulated as enzyme inhibitors.

Errore. L'origine riferimento non è stata trovata. introduces the context of the work and clarifies the objectives of this research.

Errore. L'origine riferimento non è stata trovata. extensively reports the methods adopted for materials and biocatalyst preparation along with their characterization procedures.

The initial stage of this research was studying the characteristic features of the commercial preparation of LDH-A: enzyme dimension, the influence of pH and temperature on enzyme activity, and substrate affinity. The explorative attempts were carried out to covalently immobilize the enzyme on commercial mesoporous silica. The effects on the immobilized enzyme activity and stability due to the

covalent immobilization approach adopted, the nature of the reactive groups grafted on the silica surface and the presence of a stabilizing agent were subjected to evaluation (**Errore. L'origine riferimento non è stata trovata.**).

Errore. L'origine riferimento non è stata trovata. investigates how the support's morphology, along with its chemical and physical properties, influences the activity and stability of the enzyme. Three siliceous supports were prepared and characterized. The acidity of the silica were characterized by studying the interaction with probe molecules (CO and NH_3) through FT-IR spectroscopy.

The Enzyme was subsequently immobilized on the three siliceous supports using 0.05 mg ml^{-1} of PEG or 300 mM Trehalose as stabilizing agent to protect the enzyme from the alkaline environment required for immobilization.

Errore. L'origine riferimento non è stata trovata. examines the most promising heterogeneous biocatalyst. The macroscopically distribution of the enzyme on the mesoporous silica was observed with fluorescence optical microscopy: the enzyme was labeled with a red fluorescence dye and immobilized on support. Then, FT-IR spectroscopy was used to inspect the nature of the interaction between the enzyme and the support. The kinetics behavior of the heterogeneous biocatalyst and of the free LDH-A were analyzed with no interferents, with two inhibitors (NHI-2 and Galloflavin), and with the solvent used for the inhibitory compounds. The apparent parameters calculated were used to identify the type of inhibition effect produced by NHI-2 and Galloflavin. Molecular Docking simulation was used to highlight the binding site of the inhibitors. Moreover, the stability of LDH-A in organic and alkaline solutions, together with the reusability of the heterogeneous biocatalyst was analyzed.

In conclusion, **Errore. L'origine riferimento non è stata trovata.** evaluates a three-electrode electrochemical setup as the sensing component of the proposed device, designed to measure NADH as the analyte. The apparatus was equipped with: Ag|AgCl used as the reference electrode (RE) stored in 3M KCl, a Pt wire as the counter electrode (CE), and a Ti-modified glassy carbon electrode (GCE) serving as the working electrode (WE). The enzymatic activity was followed by monitoring the amperometric signal which is correlated with the NADH concentration.