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Radiofrequency triggered enzymatic reaction inside hydrogel microparticles

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Introduction

Biochemical reactions in living organism usually take place inside cells. The characteristic feature of these reactions is their executability; the organism does not produce certain substances all the time, but only when they are needed. The reaction is usually triggered after receiving an extra- (or intra-) cellular signal. Stimuli-responsiveness is mostly possible because of compartmentalization of the cells. Reactants, enzymes and other factors are separated to different parts of cells by membranes forming organelles. Upon receiving a signal, the substances are selectively transported through the membranes and a reaction occurs.

In order to mimic that behaviour we present internally-structured cell-like micro-particles with a diameter around 50 µm. The particles are formed by an alginate gel, thermo-responsive liposomes form internal compartments filled by an encapsulated content. The particles also contain ferrofluid (Fe_2O_3 magnetic nanoparticles). High-frequency magnetic field causes heating of the particles hence release the content from thermo-sensitive liposomes into the gel body and then out of the particles (Hanuš, et al. 2013). Thermo-sensitive liposomes are characteristic by a change of bilayer structure at certain temperature.

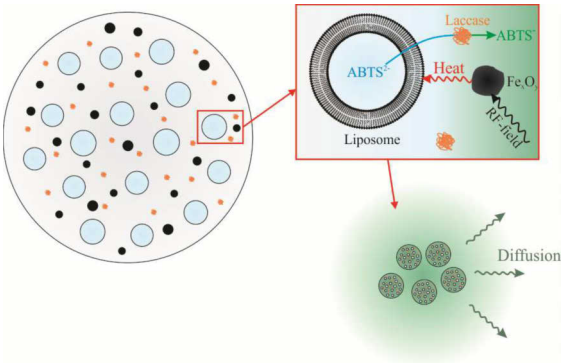

Figure 1: Scheme of the particle and principle of triggering the reaction. The substrate is released from liposomes after induction heating of particles and it is oxidized in the gel by means of the immobilized enzyme.

Utilizing these particles we studied an enzymatic oxidation of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) catalyzed by the enzyme laccase. The substrate was encapsulated inside the liposomes and the enzyme was immobilized into the alginate hydrogel. A scheme of the particle and the principle of reaction triggering is shown in Figure 1.

Preparation

Liposomes were made from DPPC (*1,2*-dipalmitoyl-*sn*-glycero-*3*-phosphocholine) and cholesterol at a molar ratio of 8:1 (DPPC:cholesterol). They were prepared by the hydration method followed by an extrusion through 100 nm polycarbonate membrane. Magnetic nanoparticles were made by the precipitation of $FeCl_2$ and $FeCl_3$ in an ammonia solution. A mixture of the ferrofluid, liposomes with ABTS, laccase and sodium alginate was dripped into a solution of copper(II) sulphate by a syringe with a needle in order to produce 1-2mm particles (Ullrich, et al. 2013) or by an ink-jet print-head in the case of micro-particles (~80 µm) (Haufová, et al. 2012). Microscopic image of such micro-particles is in Figure 2. After 1 hour of cross-linking, the particles were washed with water and then transferred to 10 mM acetate buffer (pH = 5.0). The product of the enzymatic reaction is intensively coloured; this enables spectroscopic detection at 415 nm.

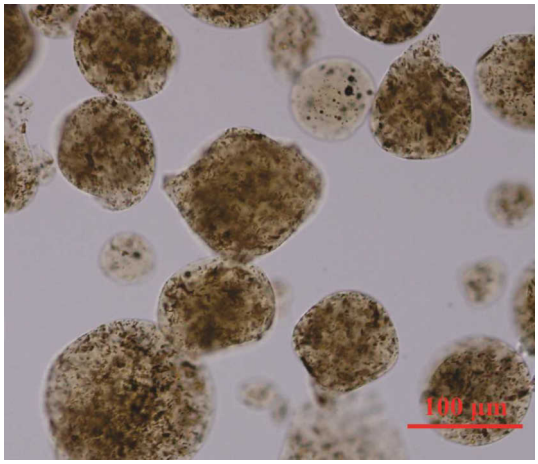

Figure 2: Microscopic image of ink-jetted micro-particles containing iron oxide nanoparticles, laccase and liposomes enclosing solution of ABTS.

Results

At first, laccase was immobilized within calcium alginate gel. A significant decrease of the enzyme activity was observed. Leakage of the enzyme during cross-linking and the presence of calcium ions was found to have the main effect on the enzyme deactivation. After 1 hour of gel cross-linking, about half of the original amount of laccase leaked out of the beads. Moreover, the activity of laccase in 150 mM $CaCl_2$ was about 10-times lower than in 100 mM acetate buffer. On the other hand, the enzymatic reaction was found to be slightly faster in 150 mM copper(II) sulphate compared to the buffer. That led us to use copper sulphate solution for cross-linking instead of calcium chloride that was used originally for the preparation.

The temperature-dependent release rate of the product has been investigated. At first, millimetre-sized particles were studied. It was found that approximately 10 % of the substrate was released at room temperature (~23 °C) in 3 hours, but the leakage could be decreased to 1 % by storage in a refrigerator. On the other hand, only 5 minutes in a 50°C water bath was enough to release and oxidize almost all of the encapsulated substrate.

Similar experiment was carried out with iron-oxide nanoparticles and RF-heating by an induction coil (frequency 400 kHz, max. amplitude 20 mT ~ 100% in Figure 3). The effect of the magnetic field intensity and duration of the RF-pulse were studied. Precise control of the magnetic field led to a step-wise release hence dosage control of the product. Functionality of this principle was then verified on micrometre-sized particles prepared by the ink-jet printing (Figure 3).

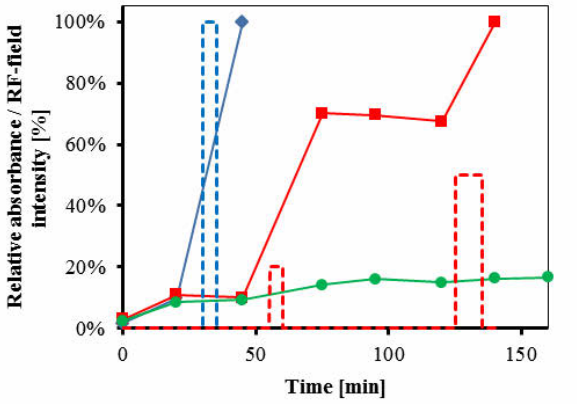

Figure 3: Release of the reaction product from alginate micro-particles. Legend: (♦)(■) samples heated by an induction coil; (●) not-heated reference sample; (- -) intensity of RF-heating. Relative absorbance 100% refers to an absorbance after release all the product.

Conclusions

Artificial cell-like particles have been created. Liposomes have a function of inner compartments and an alginate gel forms an artificial protoplasm. The particles contain also iron oxide nanoparticles enabling a control of processes inside by a temperature change. Utilizing this system we demonstrated the ability to control the rate of reaction-diffusion processes remotely by a RF-field. Micro-particles containing thermo-sensitive liposomes, holding the substrate for enzymatic reaction (ABTS), and an immobilized enzyme (laccase) released a significant amount of the reaction product after an exposure to a RF-magnetic field. This opens up the possibility to use such particles as micrometre-scaled bioreactors for more complex reaction networks using liposomes with different composition hence a different bilayer transition temperature. Also local, on-demand synthesis and release of pharmacologically or otherwise active compounds with a short lifetime could be possible.

References

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