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Rapid communication

An enzyme-modulated oxygen-producing micro-system for regenerative therapeutics

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ABSTRACT

This study suggests the idea of treating oxygen as a drug in a biological environment and demonstrates that it will exhibit a dosage-dependent trend. To accomplish this, a micro-system was fabricated, having hydrogen peroxide as the oxygen-generating source, which was decomposed using catalase, a common enzyme found in nearly all living organisms. The relevance of the proposed micro-system was justified using cell viability assays under well-controlled and fixed conditions. This study was performed under two controlled conditions, normoxia and hypoxia, and tests were carried out using three different configurations of samples under each condition: direct addition of H_2O_2, H_2O_2 encapsulated with single layer, and H_2O_2 encapsulated with double layers. This study demonstrates that the elegantly designed micro-system managed to control the decomposition of H_2O_2 and avoided direct contact with cells, while also maintaining cell viability under a low oxygen environment.

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One of the foremost challenges in the development of three-dimensional scaffolds for tissue regeneration is the adequate supply of oxygen (Harrison et al., 2007; Lysaght and Reyes, 2001; Oh et al., 2009; Vaupel et al., 1989). Cells transplanted apart from blood vessels either fail to engraft or rapidly die due to oxygen deprivation. The limited oxygen in transplanted tissue increases the risk of inflammation and infection, reduces the efficacy of medical treatments and may ultimately lead to tissue necrosis (Oh et al., 2009). Besides tissue regeneration, an inadequate supply of oxygen to tissue is also related to other medical conditions, such as bedsores, burns, and wounds, and to limiting the benefits of radiotherapy (Cavalli et al., 2009). Although this is a well known problem and a great deal of effort has been put forth to resolve it, the paradigm of recognizing oxygen as a kind of drug has yet to be established. As a result, advancement in this area is far behind that of conventional drugs containing organic molecules, and no research following the standard protocol of developing a new drug has yet been seen. This may be due to the gas nature of oxygen, which makes the delivery mechanism very sophisticated.

Research in this area has long been performed by our group, and we have recently successfully developed a polymeric micro-system that releases hydrogen peroxide and, to some extent, oxygen in a controlled manner (Ng et al., 2010). The study involved the direct encapsulation of hydrogen peroxide (H_2O_2) into a polymer matrix of poly(D,L-lactide-co-glycolide) (PLGA) via the double emulsion solvent evaporation method. This study focused on the engineering of the micro-system, using biomaterials to safely decompose H_2O_2 into oxygen, in which the oxygen is treated as an active drug for prolonging cell survival under hypoxic conditions.

In fact, decomposition of H_2O_2 was reported to be very slow in the absence of a suitable catalyst (Melnyk et al., 1979), and this slow decomposition would be problematic for practical applications, as it is suggested here for biomedical purposes.

To overcome such a hurdle, the proposed delivery system may be an appropriate method for fabricating a safe micro-system for effective oxygen production that can be used for treating oxygen deficiency. In the structural design of the micro-system, a dual layer scheme has been adopted, as illustrated in Fig. 1. In this system, the H_2O_2 was first isolated via microencapsulation within a PLGA shell. Then the microspheres were embedded into a secondary alginate matrix with catalase pre-immobilized to the chains. Catalase was adopted to accelerate decomposition of the hydrogen peroxide and was chemically bound to an alginate backbone. Alginate was chosen, as it is biocompatible and biodegradable, and has carboxylic groups for the grafting of secondary molecules of a catalase (Fundueanu et al., 1999; Giordano et al., 1993; Wu and Grainger, 2006).

In the proposed micro-system, when H_2O_2 is diffused throughout the first core layer of PLGA, some H_2O_2 will be decomposed into oxygen, while the remaining H_2O_2 will be released as free H_2O_2.

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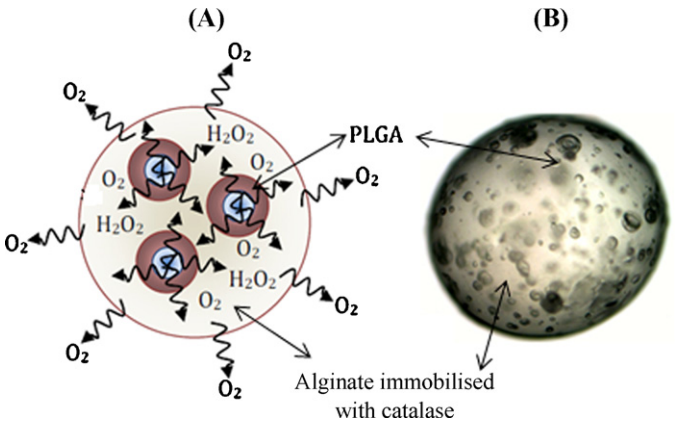

Fig. 1. (A) Theoretical decomposition route for H_2O_2 in a micro-system in releasing oxygen and (B) Optical microscope image of a PLGA-based micro-system encapsulated with alginate-catalase. Immobilization of catalase onto an alginate chain was carried out using EDC/NHS chemistry. The alginate layer coating on the PLGA micro-system was produced by the dripping method, in which the gelation of alginate was achieved using divalent calcium(II) ions.

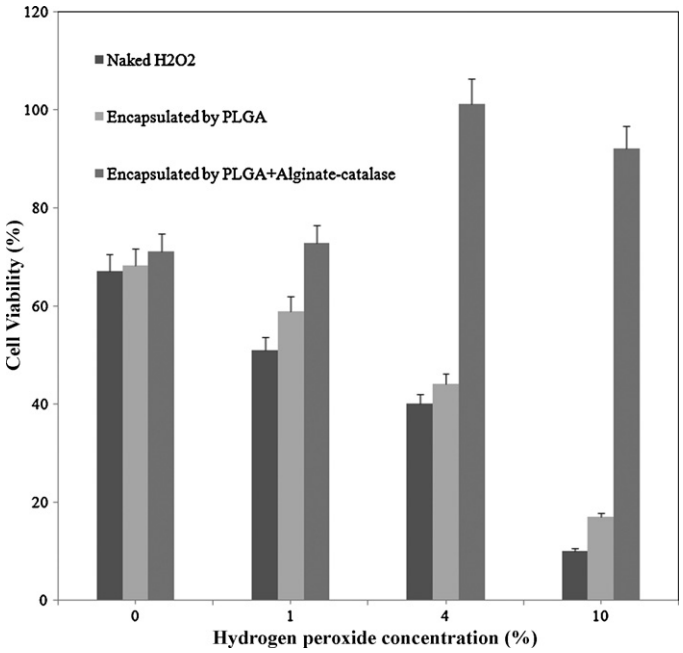

Fig. 3. The effect of H_2O_2 encapsulation inside a polymeric shell and coating with alginate catalase on cell viability under hypoxic conditions. Hypoxic conditions were created by flushing a mixture of hypoxic gases (1% O_2, 5% CO_2, and 94% N_2) using a Billups–Rothenberg modular incubation chamber. Samples were sealed before placement into a tissue culture incubator.

However, due to the presence of catalase, decomposition of H_2O_2 becomes more active and faster within the secondary layer, which minimizes the possibility of the release of free H_2O_2 from the micro-system. At the end of the route, only oxygen, which will be safe and useful for biological applications, is released into the environment.

Harrison et al. (2007) demonstrated the possibility of using peroxide-based salt to generate oxygen for tissue development. In their work, intermediate H_2O_2 was produced from the peroxide salt that had been directly incorporated into scaffolds. The supply of oxygen was obtained upon the decomposition of the H_2O_2 assisted by mobile catalase. Our work employed the direct use of H_2O_2 via encapsulation for a controlled release, in order to eliminate excess salt byproducts such as cations that can be harmful to the bio-environment. The enzyme was also immobilized to enhance its activity and to minimize the possibility of leaching, which may have side effects on the biological system.

It is known that changing the structural design parameters of a micro-system can alter the release profile of oxygen. For instance, increasing the thickness of the alginate coating generally increases the diffusion path of oxygen to the environment. Therefore, varying the concentration of alginate can be used to alter the release profile of oxygen. From the results obtained (Fig. 2), it was clear that a lower concentration of alginate showed a higher release of oxygen at the beginning of incubation. This was due to the higher porosity of the alginate layer, as the concentration was insufficiently high. As a result, oxygen was released faster and the dissolved oxygen

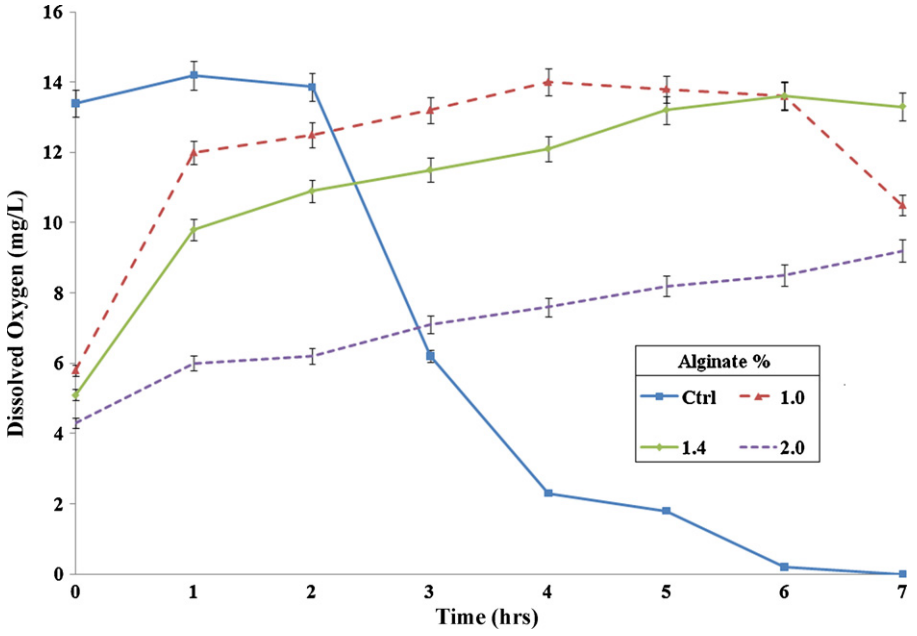

Fig. 2. Controlled release profile of oxygen formulated by encapsulation of H_2O_2 into a PLGA micro-system coated with catalase and immobilized with 1.0%, 1.4% and 2.0% alginate.

(DO) level increased. Conversely, increasing the concentration of alginate can produce a packed coating layer, resulting in slower diffusion and sustaining longer release times, as demonstrated in Fig. 2.

Cells require an optimum amount (not over or under dosing) of oxygen for survival (Malda et al., 2007). In order to investigate the proper dosage, a controlled experiment was carried out to test the effect of encapsulated H_2O_2 concentration on cell viability. Fig. 3 shows the cell viability of different sample sets having free H_2O_2 and encapsulated H_2O_2. As expected, direct contact of H_2O_2 with cells showed serious toxic effects on cell viability. The controlled release of H_2O_2 and barrier layers between the cell and H_2O_2 resulted in improved cell viability. In general, encapsulated groups revealed significantly increased viability. Because of this unique feature, the micro-system can be widely used for applications where oxygen is necessary, such as under hypoxia conditions. Since engineered tissue of a clinically relevant size requires oxygen to sustain viability until supporting vascularization forms, our system can supply oxygen during this critical time.

In conclusion, the observations recorded in this study demonstrate that an oxygen-producing micro-system has the ability to provide an adequate environment for cells under a hypoxic environment, and results in increased cell survival. This double-layered micro-system opens up the possibility of treating oxygen as a drug for treating diseases related to oxygen deficiency. Future studies are needed on the specific optimization of the micro-system targeting particular applications. For instance, in tissue regeneration, this micro-system can be used as supporting material and incorporated into scaffolds for large area tissue implants.

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