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Review

Polymeric nanoparticles for hemoglobin-based oxygen carriers

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ABSTRACT

This article reports on the current status of the research on blood substitutes with particular attention on hemoglobin-based oxygen carriers (HBOCs). Insights on the physiological role of hemoglobin are reported in the view of the development of both acellular and cellular hemoglobin-based oxygen carriers. Attention is then focused on biocompatible polymeric materials that find application as matrices for cellular based HBOCs and on the strategies employed to avoid methemoglobin formation. Results are reported regarding the use of bioerodible polymeric matrices based on hemiesters of alternating copolymer (maleic anhydride-*co*-butyl vinyl ether) for the preparation of hemoglobin loaded nanoparticles.

1. Introduction

Several diseases are related to heme and hemoglobin disorders. Gene mutations result in a group of hereditary diseases termed hemoglobinopathies, among which the most common are sickle-cell disease and thalassemia. Decreased levels of hemoglobin and heme synthesis lead to symptoms of anaemia, whereas alterations of heme metabolic pathways generate porphyrias syndromes.

Medical interest toward hemoglobin (Hb) is related to the possibility of its administration as blood substitute to re-establish oxygen homeostasis in tissues. At present, blood transfusions are mostly applied for this purpose, but the need of the right type of blood and its short shelf life are still serious problems to be overcome. In this case, the use of autologous transfusions prevents the need of cross-matching and, although autologous transfusions are considered the safest, they are not always feasible because they may cause perioperative anaemia and are more expensive than allogenic transfusions [1–3].

The development of oxygen carriers is particularly indicated in the case of urgent need of oxygen delivery to tissues and to solve the above mentioned problems related to blood transfusions. The ideal oxygen carrier would deliver oxygen, not transmit disease, not have immunosuppresive effects, would have less strict storage requirements than for human blood, would not need cross-matching, would be available at reasonable costs, be easy to administer, and able to reach all areas of human body, including ischemic tissues [4–6].

The potential clinical application of oxygen carriers covers several diseases such as sickle-cell anaemia, autoimmune hemolytic anaemia, infarction, surgery and traumas. Their administration would also be accepted in Jehovah's Witness community without violation of the doctrine on blood [7]. Furthermore, it is known from more than 50 years, that solid tumors are characterized by hypoxia. Hypoxic cell results more resistant to standard chemotherapy and radiotherapy, are more invasive and metastatic, resistant to apoptosis and genetically instable. Nowadays, some oxygen carrier products are experimentally and clinically investigated as cancer chemo- and radio-sensitizing agents [8].

2. Oxygen carriers

Hemoglobin (Hb) is the physiological oxygen-transport metalloprotein present in red blood cells, in mammals and other animals. Hemoglobin primary function is to bind oxygen that diffuses into the bloodstream from the lungs, transport it to outlying tissues and release it mainly for aerobic respiration. Hemoglobin molecule has a tetrameric structure (64 kDa), made of two α and β globin chains. Each unity has a molecular weight of 16 kDa and contains a prosthetic moiety. The prosthetic group is constituted by an iron atom conjugated to a porphyrinic ring (heme), buried in a hydrophobic pocket, and capable of carrying one oxygen molecule [9].

During the, fetal, neonatal and adult stages of life, two main forms of hemoglobin which differ in globin sequence have been identified: in

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adults 96–98% is hemoglobin A, while during embryonic life and early neonatal period hemoglobin F, which has a higher oxygen affinity, is mainly present [10]. Following the two-state model of Monod et al. [11] hemoglobin can be mainly in two conformations, different by a tridimensional point of view. The two conformations, called T and R, are strictly related to oxygen binding to heme group. While the deoxy protein prefers a 'tense' state (T), the binding of the ligand determines the shift of the allosteric equilibrium towards the relaxed state (R) [12]. OxyHb and deoxyHb differ in the UV/VIS spectrum of absorbance, which are commonly used for the characterization of hemoglobin samples or artificial hemoglobin-based oxygen carriers.

The binding of oxygen by the protein is cooperative: as hemoglobin binds successive oxygen molecules, the oxygen affinity of each subunit increases. This is caused by changes in the tetrameric structure of the protein after the binding of the ligand. This behavior is also reflected into a sigmoidal, rather than hyperbolic oxygen dissociation curve.

The cooperative binding of oxygen to hemoglobin is usually quantified by applying the Hill equation [13] (Eq. (1)).

$$\log\frac{[\theta]}{[1-\theta]} = n\log pO_2 - n\log p50 \quad (1)$$

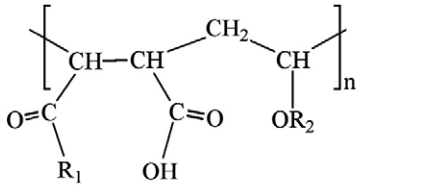
In this equation n represents the Hill coefficient, while $p50$ is the oxygen tension needed to saturate 50% of binding sites. The Hill coefficient reflects the cooperative activity between hemoglobin subunits. Hill coefficient of 2.7 reflects a cooperative tetrameric hemoglobin; otherwise, for a non-cooperative hemoglobin Hill coefficient value is 1 [9].

The link between hemoglobin and oxygen is essential for the protein homeostatic function. Anyway, hemoglobin can be easily oxidized by oxygen itself to methemoglobin (Fe^{2+} to Fe^{3+}) generating the superoxide anion [14] and other reactive oxygen species (ROS). The spontaneous oxidation of Fe^{+2} is called autoxidation and within the red blood cells is generally maintained at 3% of total Hb by metHb reductase system, including superoxide dismutase and catalase.

In the early seventies, the unfeasibility to administer free hemoglobin (Hb) as oxygen therapeutic due to its short half life, abnormal high oxygen affinity and the incoming of serious side effects, namely malaise, abdominal pain, hemoglobinuria, and renal toxicity was observed [15]. The toxic effects of hemoglobin infusion were correlated to the breakdown of the tetramer into dimers in the bloodstream, each dimer containing an α and a β subunit [4].

As alternatives to the administration of free hemoglobin, the use of synthetic oxygen carriers based on fluorocarbons (FBOCs) or hemoglobin-based oxygen carriers (HBOCs) has captured the attention of scientists [16–21].

FBOCs are oil-like chemicals presenting high gas solubility. As reported by Clark and Gollan [22] a mouse can sustain life if submerged in a FBOC solution, equilibrated with oxygen. To be used as blood substitutes, FBOCs are generally emulsified in aqueous media using different surfactant agents in order to improve their dispersion in plasma-like solutions. Anyway, the presence of severe side effects derived by the use of FBOCs (reduction in platelet activity, and immunogenic response [23]) determined an increasing attention of the scientific community in developing both acellular and cellular HBOCs systems.



R_1: $O(CH_2)_2OCH_3$; PEG (MW 2000Da)

R_2: $(CH_2)_3CH_3$

Fig. 1. 2-Methoxyethyl hemiester of poli(maleic anhydride-*alt*-butylvinyl ether) 5% grafted with methoxy-PEG2000.

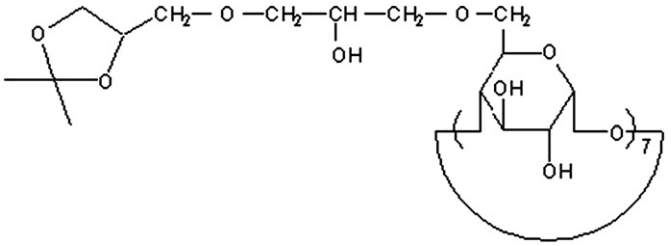


Fig. 2. *O*-glycidyl-*O*-isopropyldenglycerol β-cyclodextrin.

2.1. Acellular hemoglobin-based oxygen carriers (HBOCs)

Acelullar HBOCs consist of solutions of purified Hb, usually modified in order to avoid the breakdown of the tetramer into the bloodstream. The acellular HBOCs include intramolecularly cross-linked Hb [24,25], polymerized Hb [26,27], polymer-conjugated Hb [28,29], and recombinant cross-linked Hb [30]. Cross-linking of Hb was investigated also for the preparation of stable microcapsule made with hemoglobin itself. Terephthaloylchloride was applied as cross-linking agent, allowing for the incorporation of inositol hexaphosphate and glucose, and followed by stabilization through glutaraldehyde. In this work, the 5-μm diameter microcapsules were able to ensure oxygen transfer, but suffered rapid lysis by proteases [31].

Generally, chemical modifications on Hb molecule are associated to a low oxygen affinity and changes in the cooperativity and in the binding to carbon dioxide and chloride, which are normal allosteric modifiers of Hb oxygen affinity [32,33]. Moreover, the major side effects reported in the case of the administration of acellular HBOCs are their action as plasma expander and induced hypertension [34–37]. It has been assumed that modified Hb could easily enter into the interstitial space and there acts as a sink in binding and removing the nitric oxide needed for maintaining the normal tone of smooth muscles [21].

Non-hypertensive effect on Hb solution can be generated by enhancing the molecular size of the protein [38]. Hemoglobin pegylation is considered a promising way to achieve this objective [39,40]. The

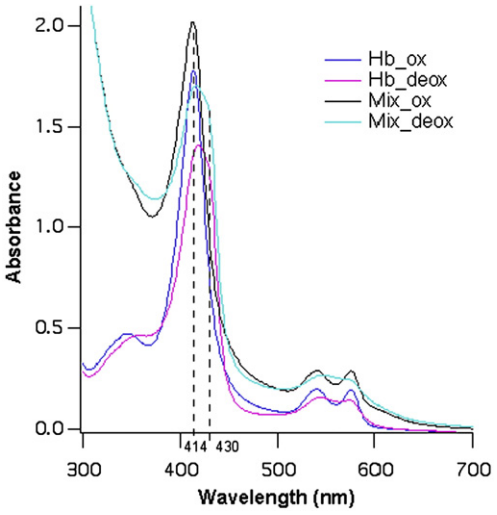


Fig. 3. UV-VIS spectra of Hb solution (Hb) and Hb/polymer/stabilizer mixture (Mix) saturated with air, about 21% O_2 (_ox), or partially deoxygenated under N_2 (_deox). Hb-oxygenated λ_{max}: 414 nm; Hb-deoxygenated λ_{max}: 430 nm.