Polyethylene Glycol Microspheres Conjugated with Hemoglobin as Artificial Red Blood Cells

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Introduction: In the US, 5 million people require blood transfusions each year. Although generally safe, there are drawbacks to blood transfusions including fever, acute immune or delayed hemolytic reactions, anaphylactic reactions, transfusion related acute lung injury, and bloodborne infections. Despite screening for diseases such as HIV and hepatitis, the risk of contraction is nonzero, and there are continually emerging bloodborne diseases such as Zika that are not yet screened for. Additionally, there are often blood bank shortages. These complications have driven decades of research into artificial blood, yet to date there are no blood substitutes clinically available. While hemoglobin based oxygen carriers have shown promise, they also show oxidative damage to tissues, particularly in cardiac and renal tissues. Both high and low oxygen PEGylated hemoglobin (Hb) have shown such oxidative stress. We hypothesized that this oxidative stress was due to direct delivery of the PEGylated Hb and conjugated PEGylated Hb onto PEG hydrogel microspheres. In this study, we probed the ability of the Hb microspheres to deliver oxygen.

Materials and Methods: Hemoglobin (10 µg) was reacted overnight with acrylate-poly(ethylene glycol)-succinimidyl valerate at a 1:1.06 (Hb: PEG-SVA) molar ratio in HEPES buffered saline with 20 mM n-(2-Hydroxyethyl)piperizine-N′-(4-butanesulfonic acid), 100 mM sodium chloride, 2 mM calcium chloride, and 2 mM magnesium chloride at pH 8.5. to form acrylate-PEG-Hb (PEG-Hb). Microspheres were formed by combining PEG-Hb (0.15625 µmol/mL) with polyethylene glycol diacrylate (PEGDA; 10 % w/v), HEPES buffer solution, eosin Y (1% w/v), triethylolamine (1.5% w/v), and 3.75 µL/mL of a photoinitiator solution containing 2,2-Dimethoxy-2-phenylacetophenone in 1-Vinyl-2-pyrrolidinone (300 mg/mL) then adding this precursor polymer solution to a mineral oil solution supplemented with the photoinitiator (3 µL/mL) and photopolymerizing a vortex-induced emulsion under white light for a minimum of 1 minute. Microspheres were harvested through centrifugation. Microsphere capacity to carry oxygen was evaluated by measuring dissolved oxygen levels of four test groups: 70 mL of phosphate buffered saline (PBS) x 3, or deionized water (DIW). Prior to testing, groups were stored overnight under argon while microspheres were exposed to atmosphere. The probe was slowly inserted into the jar, as to not displace the argon, and data points were collected at different time intervals for the controls with no microspheres. Microspheres suspended in PBS (100 µL/mL) were added to 70 mL samples in increments of 500 µL for a total addition of 2 mL of microsphere suspension (200 µL microspheres). Resulting microspheres were imaged and analyzed with NIH ImageJ.

Results and Discussion: The 160 and 280 s timepoints at which the hemoglobin microspheres were added to solution resulted in an 18% and 20% increase of the oxygen content of the solution, respectively. Conversely, the greatest increase in the empty microsphere's oxygen content was 10%, and was due to the increased oxygen content of the solution. The empty microspheres ranged in diameter from 5.4 - 78.7 µm while the hemoglobin microspheres ranged in diameter from 13.1 – 132.6 µm.

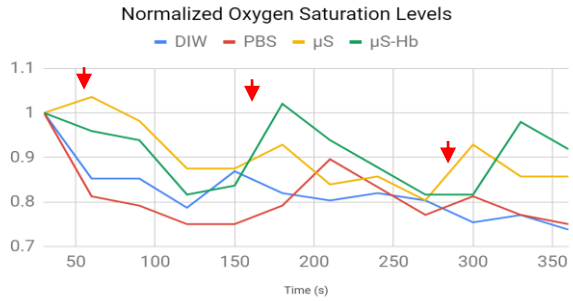

Figure 1. Oxygen levels of deionized water (DIW, blue), PBS (red), empty microspheres (µs, yellow), and microspheres with bound hemoglobin (µs-Hb, green). Red arrows show time points at which 500 µL of microspheres were added to PBS.

Conclusions: PEGylated hemoglobin conjugated to PEGDA microspheres can function as an oxygen carrier. Future steps are to evaluate their oxidative stress in tissues. Although emulsion methods are capable of generating microspheres on the size order of red blood cells, moving towards microfluidics would give more consistency.

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References: 1. Ronda, Luca, et al. Free Radic Biol Med 124 (2018): 299-310.