Synthesis of Stroma-Free Haemoglobin Nanoparticle as an Artificial O_2 Carrier with Antioxidant Ability

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The shortage of blood supply caused by declining birth-rate and aging population has become a social problem worldwide. To compliment transfusion therapy, red blood cell (RBC) substitutes must be developed quickly. Although various types of haemoglobin (Hb)-based O_2 carriers (HBOCs) have been manufactured, they have not yet been put into practical use.[1] The decrease in O_2 transporting capacity associated with the autoxidation of Hb remains one of the unsolved problems. Stroma-free haemoglobin (SFHb) obtained from human RBC contains antioxidant enzymes, catalase (Cat), that remove reactive oxygen species. In this paper, we report the synthesis of SFHb nanoparticle (SFHbNP) composed of polymerized SFHb covered with human serum albumin (HSA), and clarified its structure, O_2 binding property, and antioxidant ability.[2] In addition, the blood retention was evaluated in animal experiments.

A cross-linking reagent, *N*-succinimidyl-3-maleimidopropionate (SMP), was reacted with the Lys residues of SFHb, and the obtained maleimide-activated SFHb was polymerized using dithiothreitol (DTT). Subsequent covalent wrapping of the sphere with HSA yielded SFHbNPs (**Figure 1**). The diameter was determined as ca. 90 nm by dynamic light scattering. The spherical shape was clarified by scanning electron microscopy. The O_2 affinity (P_{50}) of SFHbNP was 7 Torr (pH 7.4, 37 ºC), and Hill coefficient (n; the degree of cooperativity) was 1.4. This high O_2 affinity compared to RBC could be suitable for O_2 transport to hypoxic tissues during haemorrhagic shock. As expected, SFHbNP containing a small amount of Cat in the core forms very stable O_2-adduct complex. The Hb oxidation rate in aqueous H_2O_2 solution (20 μM) after 2 hr was only 5%.

The SFHbNP solution showed good compatibility with blood components. The elimination half-life ($T_{1/2}$) in bloodstream in rats was ascertained as 20.7 hr, which is 30-fold longer than that of naked Hb. All results indicate SFHbNP as useful alternative material for RBC and O_2 therapeutic reagent in diverse medical situations.

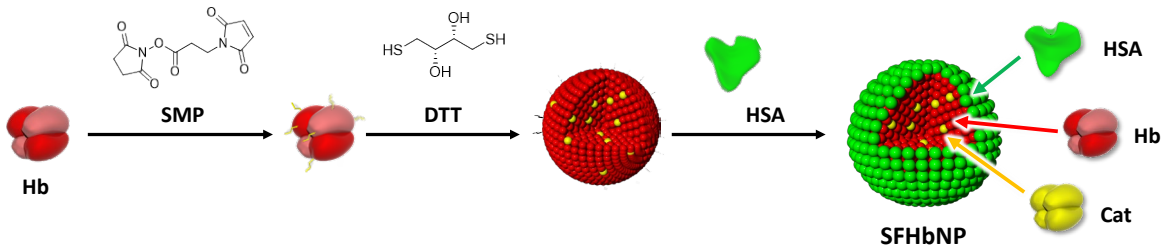

Figure 1: Synthetic scheme of SFHbNP.

References:

[1] Gupta, A. S. *Shock* **2019**, *52*, 70−83.

[2] Okamoto, W.; Hasegawa, M.; Kohyama, N.; Kobayashi, T.; Usui, T.; Onozawa, H.; Hashimoto, R.; Iwazaki, M.; Kohno, M.; Georgieva, R.; Bäumler, H.; Komatsu, T. *ACS Appl. Bio Mater.* **2022**, *5*, 5844–5853.