Hemoglobin based Oxygen Nanocarriers: An exogenous supply of O_2 for applications in photodynamic therapy

Alejandro Fábrega Puentes[1], **Paolo di Gianvincenzo**[1], **Hans Bäumler**[2], **Yu Xiong**[2], **Sergio Moya**[1]

[1]CIC biomaGUNE, Paseo Miramón 194, (Donostia-San Sebastián, Spain)
[2]Institut für Transfusionmedizin der Charité Universitätmedizin, Charitéplatz 1, (Berlin, Germany)

afabrega@cicbiomagune.es

Photodynamic therapy (PDT), a minimal invasive technique for cancer treatment, is based on generation of reactive oxygen species (ROS) in tumor tissue by the activation of O_2 through light pulses[1]. The energy of light is transferred to O_2 by photosensitizing molecules (PS). The increase of ROS results in the activation of apoptotic pathways in tumor tissue, activates the immune system and may induce the collapse of tumor vasculature[2].

One of the main drawbacks of photodynamic therapy in cancer treatment is the limited O_2 content in tumour tissue, which limits the ROS production. The use of hemoglobin (Hb), the natural O_2 carrier in blood stream, could be an interesting approach for oxygen supply to the tumor during PDT[3].

However, the use of native protein as exogenous oxygen carrier at the therapeutic level is highly conditioned since, free Hb can lead to vasoconstriction and renal injuries[3] . Hemoglobin based Oxygen Nanocarriers (HOBC) prepared by entrapping hemoglobin in polymeric or protein nanoparticles could provide a means to transport oxygen to tumor tissue without the drawbacks from free Hb delivers[4]. Also, HOBCs size should be below of 500 nm for optimal tumor penetration and reduced immunogenic response[5]. Established methodologies for HOBCs synthesis result in particles with sizes over 800 nm with limited tumor penetration.

In this framework our aim is the synthesis of small-size HOBCs that could be applied for tumor targeting. For this purpose, different procedures have been designed to obtain Hb nanoparticles (Hb-NPs). Synthesis protocols were first stablished with Bovine Serum Albumin NPs (BSA-NPs) as a model protein NPs.

Two different approaches were tested for protein NPs fabrication. **Co-precipitation** with carbonate templates and **polymer complexation**.

Co-Precipitation

Crosslinked particles: Based on the CCD[6] procedure, protein is co-precipitated with a carbonate template (MnCO3) and the amine groups are crosslinked. Subsequently the template is dissolved, resulting in a protein particle(Figure 1). Once optimized this synthesis procedure and purification steps, we were able to produce BSA particles crosslinked with size of 200-400 nm.

Layer By Layer (LBL): In this approach, after we performed the coprecipitation stage a multilayer coating of polycations and polyanion was assembled on top of the precipitates. By doing so, protein structure was not altered after the carbonate template dissolution without using crosslinking. With this procedure BSA-NPs were produced with size of 300-350 nm (Figure 1).

Polymer complexation

Protein is complexed with polyelectrolytes through electrostatic interactions. Different polyelectrolytes were tested and both proteins (Hb and BSA) were complexed in nanosystems with size of 30-50 nm (Figure 2).

Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), UV-Visible Spectra, Circular Dichroism (CD) were applied for an in-deep characterization of physico-chemical properties of our systems,

In conclusion, the nanosystems designed could be the basis, for the implementation of PDT by exogenous oxygen supply.

References

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Figures

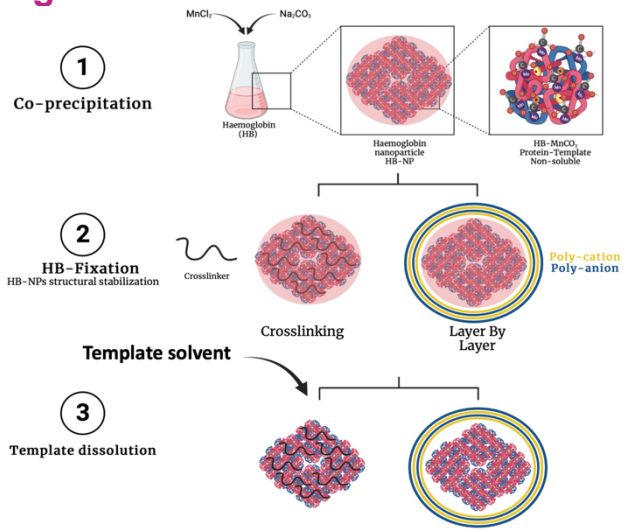

Figure 1. Scheme of synthesis of HOBCs by co-precipitation processes. Crosslinked Hb NPs (left) and NPs with LBL coating.

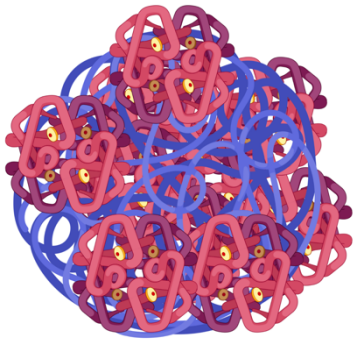

Figure 2. 1) Scheme of HOBCs produced by Polymer complexation of Hb.