論 文 の 内 容 の 要 旨

論文題目　Development of micro-sized perfluorocarbon-based oxygen carriers using SPG membrane emulsification technique（SPG膜乳化法を用いたマイクロサイズのパーフルオロカーボン酸素運搬体の開発）

氏　　名　傅　暁廷

Chapter 1 Introduction

This chapter is the background review and objectives of this research. Oxygen carriers (OCs) are expected to improve oxygen supply in the biomedical fields for targeting clinical treatments or for facilitating the growth of cells or tissues. Being one of the promising tools, OCs have been gained much attentions and proceeded for several decades. By illustrating the necessity of sufficient oxygen supply and the usefulness of OCs, two typical types of OCs were introduced: Hemoglobin (Hb)-based and perfluorocarbon (PFC)-based.

The development of Hb-based OCs with chemical modification and encapsulation was reviewed first of all. The essence of Hb and achievements of Hb-based OCs were stated, together with the concerns of using Hb as the material of OCs (e.g. risk of infection, metHb formation, limited source). Then, the coming introduction of PFC highlighted the properties and advantages of using PFC as the material of OCs. The following review on the reported PFC-based OCs presented the progress and unsolved points in this field. By exampling the potential cellular uptake via endocytosis of nano-sized OCs, micro-sized OCs were focused and the advantages of size-control and deformability were highlighted, especially some promising deformable OCs were reviewed for clearly illustrate the position of research. Finally, the introduction of Shirasu porous glass (SPG) membrane emulsification technique showed its feasibility for solving the problems in the development of OCs.

Based on these, this research is aimed at the development of micro-sized PFC-based OCs using SPG membrane emulsification technique. Firstly, a size-controlled preparation of micro-sized PFC emulsions will be presented. Next, encapsulation of PFC by biodegradable polymer to improve the stability and induce deformability will be developed.

Chapter 2 Preparation of PFC emulsions as oxygen carriers

This chapter developed a size-controlled preparation of micro-sized PFC emulsions as OCs using SPG membrane emulsification. The methodology enabled to precisely manipulate the size of emulsions via changing membrane pore size and fabricate emulsions that were similar in size to red blood cells (RBCs). Also, the size control was manipulated by type and concentration of surfactants (Pluronic block copolymer). The behaviors of surfactants during the formation of emulsions suggested that the size distribution was sensitive to the type and concentration of surfactants. In the thermal and long-term stability tests, it was found that the micro-sized PFC emulsions stabilized with Pluronic F127/F68 were highly stable over a wide span of temperatures, even at temperature for autoclaving sterilization, the F127- and F68-stabilized emulsions were also stable during storage for several months. Furthermore, functionalizing PFC emulsions to indicate oxygen level by loading Ru(ddp)-the fluorescence intensity of which corresponds to the amount of surrounding oxygen-was conducted at the surfaces of emulsions. In the oxygen loading and release experiment, the emulsions showed higher oxygen loading and release capacity than pure solvent, suggesting the function of oxygen supply. Finally, improved oxygen supply was presented by culturing emulsions with EGFP-HeLa cells that show fluorescence in response to the hypoxic condition. Through this chapter, we disclose the feasibility of SPG membrane emulsification in the development of PFC-based OCs and the PFC emulsions could be promising OCs.

Chapter 3 Preparation of PFC-filled polymer microcapsules as oxygen carriers

In order to improve the mechanical property and introduce the investigation on deformability, PFC-filled polymer microcapsules (FCs) were prepared in this chapter. The FCs were successfully fabricated using SPG membrane emulsification and evaporation-induced phase separation. The characterizations showed that formed FCs had core-shell structure and remained the PFC liquid inside. It was feasible to precisely control the size of FCs and fabricate FCs that were similar in size to RBCs. Upon enhancing the mechanical stability by the encapsulation, the use of different polymer concentrations gave rise to the controllable shell-thickness of FCs, which translated into tunable deformability. By conducting the compression tests, it was confirmed that manipulation of the type or concentration of polymers induced the tunable deformability. There was a shell-thickness dependency with deformability and FCs with soft shell (DFCs) showed more deformable than other particles. A microchannel system mimicking vascular capillary was applied to evaluate the ability to pass through confinement. Results showed that the more deformable DFCs were more permeable than other particles, and RBCs with different crosslinked contents were used as references. The current FCs were able to be size-stable over being stored for a certain period before uses. Owing to the existence of PFC, higher oxygen release capacity was found in FCs than pure water. An important portrait of the

current FCs is tunable size and deformability, enabling them to cover the size range of all the blood cells and to be models of diseased RBCs as referring with crosslinked RBCs in the permeation experiment. Therefore, multiple-use of the FCs is expected, not restricted in the oxygen supply only, models for diseased RBCs or other blood cells are also able to be accomplished.

Chapter 4 Preparation of concave-shaped deformable PFC-filled microcapsules as oxygen carriers

A new type of PFC-based OCs, concave-shaped deformable FCs (cDFCs), has been developed in Chapter 4 using the solvent-induced shape change method. Characterizations showed that the concave shape was formed in cDFCs, confirming the similarity in both size and shape to RBCs. Compression and atomic force microscope (AFM) showed that cDFCs were more deformable than some of the crosslinked RBCs and had similar Young's modulus with slightly-crosslinked RBCs. To mimic the behavior of RBCs passing through vascular capillaries, microchannel permeation experiment was performed and cDFCs were found to be more permeable through the microchannel than other particles including DFCs, and more importantly, were close to natural RBCs. Moreover, cDFCs had higher oxygen loading and release than oxygen-poor medium (PBS), the storage stability was found during the preservation of cDFCs in different media (PBS, DMEM, saline) for a certain period. The biocompatibility and enhanced oxygen supply for cells were confirmed, suggesting the promising applications as OCs. Their functions as OCs or even drug carriers are highly expected. On the other hand, an investigation of the shape change based on a shape change diagram was shown. The possible mechanism of the shape change process was speculated and the effects of isopropanol concentration and temperature on the process were discussed.

Chapter 5 Conclusions and future perspectives

This Ph.D. thesis developed the methodology of preparing micro-sized PFC-based OCs using SPG membrane emulsification technique. Several efficient OCs including new type were multi-functional and enhanced oxygen supply was confirmed. Compared with the previous studies, this thesis showed new and effective controllability on the size and deformability of the OCs and their subsequent functions. The accomplishments of the thesis are not only located in biomedical fields but also colloids and material areas. The results and discussion were significant for the development of OCs, disease diagnosis and drug carriers. The achievements also pave the roads to mimic healthy red blood cells. It is therefore concluded that this thesis has largely devoted to the development of bioengineering.