Gas Bioengineering of Artificial Red Cells

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1. Research background in Waseda Univ.

Blood transfusion systems have greatly benefited human health and welfare. Nevertheless, some problems remain: possibility of infection, blood type mismatching, immunological response, and short shelf life which is insufficient for stockpiling for emergency situation. Realization of artificial red cells (artificial O_2 carriers) is expected to solve such problems. During the long history of development of hemoglobin (Hb)-based O_2 carriers, many side effects of Hb molecules have become apparent. They imply the physiological importance of the cellular structure of red blood cells (RBCs). In Waseda, we have developed Hb-vesicles (HbV) as artificial O_2 carriers that encapsulate concentrated Hb solution with a thin lipid membrane (see attached Figs). The *in vivo* safety and efficacy of HbV have been studied extensively (*CCM* 2004; *Transfusion* 2006; *JIM* 2008). The results illustrate the potential of HbV as a transfusion alternative and promise its use for other clinical applications that remain unattainable using RBC transfusion. On the other hand, it is widely accepted that trace amounts CO and NO, known as toxic gaseous molecules, are endogenously produced for vasorelaxation, cytoprotection, etc. Administrations of exogenous NO and CO are clinically approved. Because Hb binds not only O_2, but also CO and NO, it is expected that HbV might be utilized to deliver such gases for pharmacological effects (*JBC* 2008, *Shock* 2009).

2. Aims and details of the research

The aim of our research is to establish "gas bioengineering", new methods to administer and regulate gaseous molecules (O_2, CO, NO) into pathological conditions and *ex vivo* tissue engineering using artificial red cells for versatile clinical applications.

A) To find new clinical applications of HbV as an artificial O_2 carrier (such as a perfusate for organ transplant or three-dimensional tissue engineering) by utilizing the unique physicochemical properties such as small particle size (250 nm), and adjustable O_2 affinity and rheological properties for tailor-made artificial red cells.

B) To clarify the pharmacological effect of HbV as CO and NO carriers in synergy with O_2 carrying capacity, and its mechanism in ischemia-reperfusion injury and other pathological models, where reactive oxygen species are produced or peripheral blood flow is deteriorated. Additional non-enzymatic reaction mechanisms will be established in HbV to eliminate superoxide and peroxynitrite, and to reduce metHb. Nitrite reductase–like activity to produce NO and superoxide dismutase-like activity will also be interesting to improve the pharmacological effects

C) To promote HbV as a transfusion alternative not only in South East Asia, but also in South Asia, and Middle East Asia, where the present transfusion system is insufficient, or where natural disasters or new pathogens threaten the present medical system.

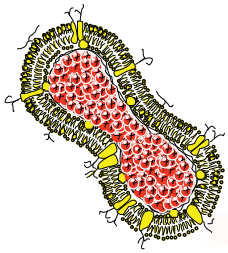
Our present academic research consortium includes researchers of Keio Univ., Red Cross, Kumamoto Univ., etc. in Japan, and UCSD, MGH, Univ. of Berne, McMaster Univ., etc. The collaborative research in Singapore; the Hub of Asia where bio-related research is extensively promoted, is expected to facilitate the worldwide development for eventual realization.

3. Experience artificial red cells from A to Z in WABIOS

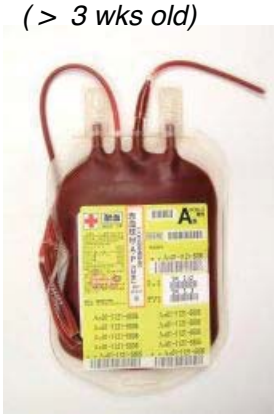
All the equipments necessary to prepare tailor-made HbV for *in vivo* use will be installed in WABIOS. After the physicochemical characterization and adjustment of the HbV suspension, it will be intravenously injected into animal models or utilized for other purposes such as a perfusate in tissue engineering. WABIOS will provide unique opportunity to study from the preparation of HbV to the efficacy and safety evaluation HbV in animal experiments. The physicochemical properties of HbV can be modified and optimized anyhow because HbV is a molecular assembly.

I have worked on this subject since 1991. The research field expands to bioengineering, biochemistry, polymer science, chemical engineering, biorheology, hematology, critical care medicine, pharmacolody, biology, tissue engineering, etc. We welcome you of any fields, an active researcher in Singapore and other oversea countries, to join our meaningful and challenging research project that will obviously benefit medical technology in the future.

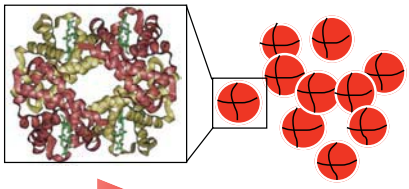
From Biological Red Cells to Artificial Red Cells



NAT inspected, Outdated RBCs
(> 3 wks old)

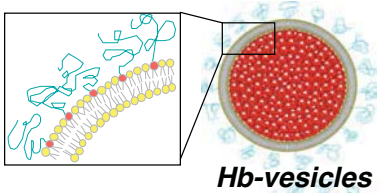


RBCs (lipids, carbohydrates, proteins, and Hb) with **residual plasma, WBC, PLT,** etc.



Purified Hb
(pasteurization / ultrafiltration)

Blood type free
Pathogen free
High purity and concentration



Hb-vesicles (HbV)

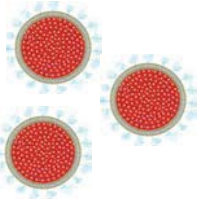
Encapsulation
PEGylation

Stockpiling at r.t. for 2 yrs

Protein Expr Purif 1993; *Biotechnol Progr* 1996; *Langmuir 1996,* 2007; *Bioconju Chem* 1997, 2000, 2004, 2009; *Biomaterials* 2004; *JBC* 2008; *BBA* 2008; *Am J Pathol* 2001; *Am J Physiol Heart* 1999, 2000, 2002, 2003, 2005, 2009; *Transfusion* 2006; *Crit Care Med* 2004,2007; *J Pharmacol Exp Ther* 2003, 2004; *JBMR* 1998, 2009; *Biomacromolecules* 2009; *J Control Release* 2009; *Shock* 2009

Gas Bioengineering of Artificial Red Cells for Potential Clinical Applications

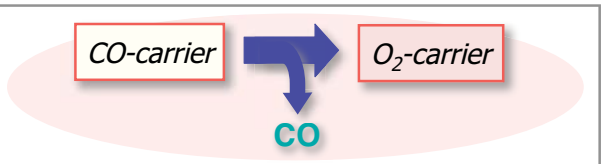
Transfusion alternative
1. Resuscitative fluid for hemorrhagic shock
2. Perioperative infusion at hemorrhage
3. Prime for extracorporeal circulation



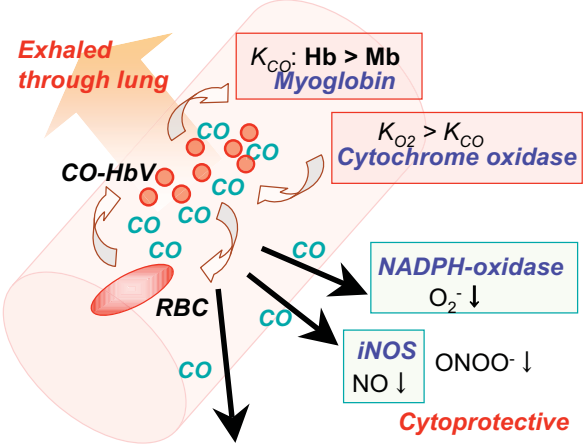
Control of O_2 affinity
Control of rheology
Design of HbV cocktail

New clinical applications
1. Perfusate for organ transplantation
2. Oxygenation of ischemic tissues
3. Oxygen supply for tissue reconstruction

And others..



Proposed mechanism of cytoprotective effect by CO-HbV



Shock 2009