# Artificial Blood Substitutes

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LifeServe BLOOD CENTER

Your Blood. Your Hospital. Your Neighbors.

# Preface

- Introduction
- Substitute Forms
- Advantages and Limitations
- Future Potential
- Summary

# Introduction

- Artificial blood or blood surrogates are substances used to mimic and fulfill some functions of biological blood for oxygen carrying a capacity
- The main function is to provide an alternative to blood transfusion
- This can be developed in different ways using synthetic production, chemical isolation, or recombinant technology

# History

- 1616-William Harvey described blood in circulation throughout the body
- Medical practitioners experiment with numerous substances such as beer, urine, milk, plant resins, and sheep's blood as a substitute for blood
- 1665- Richard Lower in Oxford conducted the 1st successful canine transfusion
- 1667- Jean–Baptiste Denis reported successful sheep-human transfusion
- 1678- Animal-human transfusions ban in France because of poor results
- 1818- James Blundell performs the 1st successful documented human transfusion in female suffering postpartum hemorrhage; received blood from her husband and survived

# History

- 1854- Patients were injected with milk to treat Asiatic Cholera; physicians believed the milk helped regenerate WBCs
- 1883- Ringer solution developed as a volume expander (Sydney Ringer)
- 1939- The Rhesus system was identified and recognized as the major cause of transfusion reactions
- Mid-20$^{th}$ Century - WWII and Vietnam war ignite in the search for blood substitutes- (hemoglobin solutions and synthetic O2 carriers)
- 1989- The 1$^{st}$ oxygen carrying blood substitute called Fluosol DA-20, a perfluorocarbon-based product, was manufactured in Japan

# Need for Blood Substitutes

- Increased demand with decreased supply
- Short shelf life: Leading to large amounts of wastage of blood products
- Seasonal blood shortages
- Difficulty finding available blood products for patients with rare blood groups
- Immunologic incompatibility
- Decreased number of available, compatible donors
- Increased risk for disease transmission: HIV, HBV, HCV
- Cost
- Risk of transfusion reactions- TRALI, AHTR, DHTR, TACO, etc

# The Ideal blood substitute will…

- Oxygen carrying capacity, equaling or surpassing that of biological blood
- Volume expansion
- Universal compatibility: Elimination of crossmatch
- Pathogen free
- Minimal side effects
- Has the ability to survive over a wide range of storage temperatures
- Long shelf-life
- Should be cost efficient

# BLOOD SUBSTITUTES INCLUDE:

*1. Plasma volume expanders or

*2. Replicate the oxygen carrying function of natural blood

1. Plasma expanders include:
   crystalloids, colloid
   gelatin, dextran, hydroxyethyl starch
   albumins

**Problems with plasma expanders include anaphylaxis, disease transmission, nephrotoxicity, electrolyte imbalance, and volume overload**

# 2. Red Blood Cell Substitutes

- 1. Perfluorocarbons
- 2. Hemoglobin- Based Oxygen Carriers
- 3. RBC differentiated from stem cells

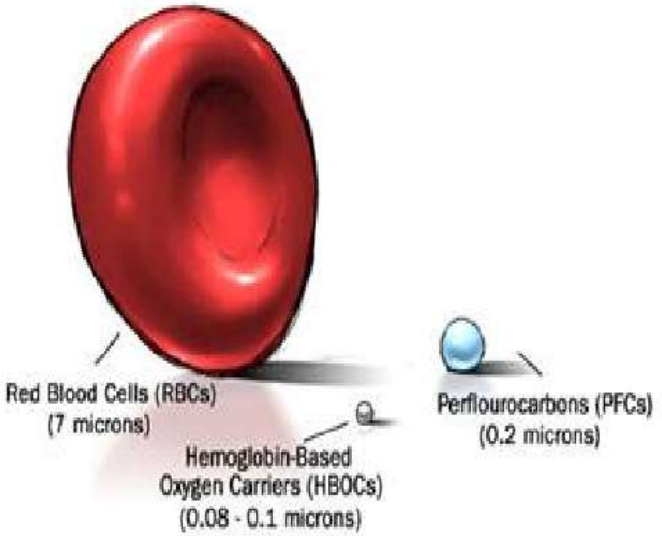
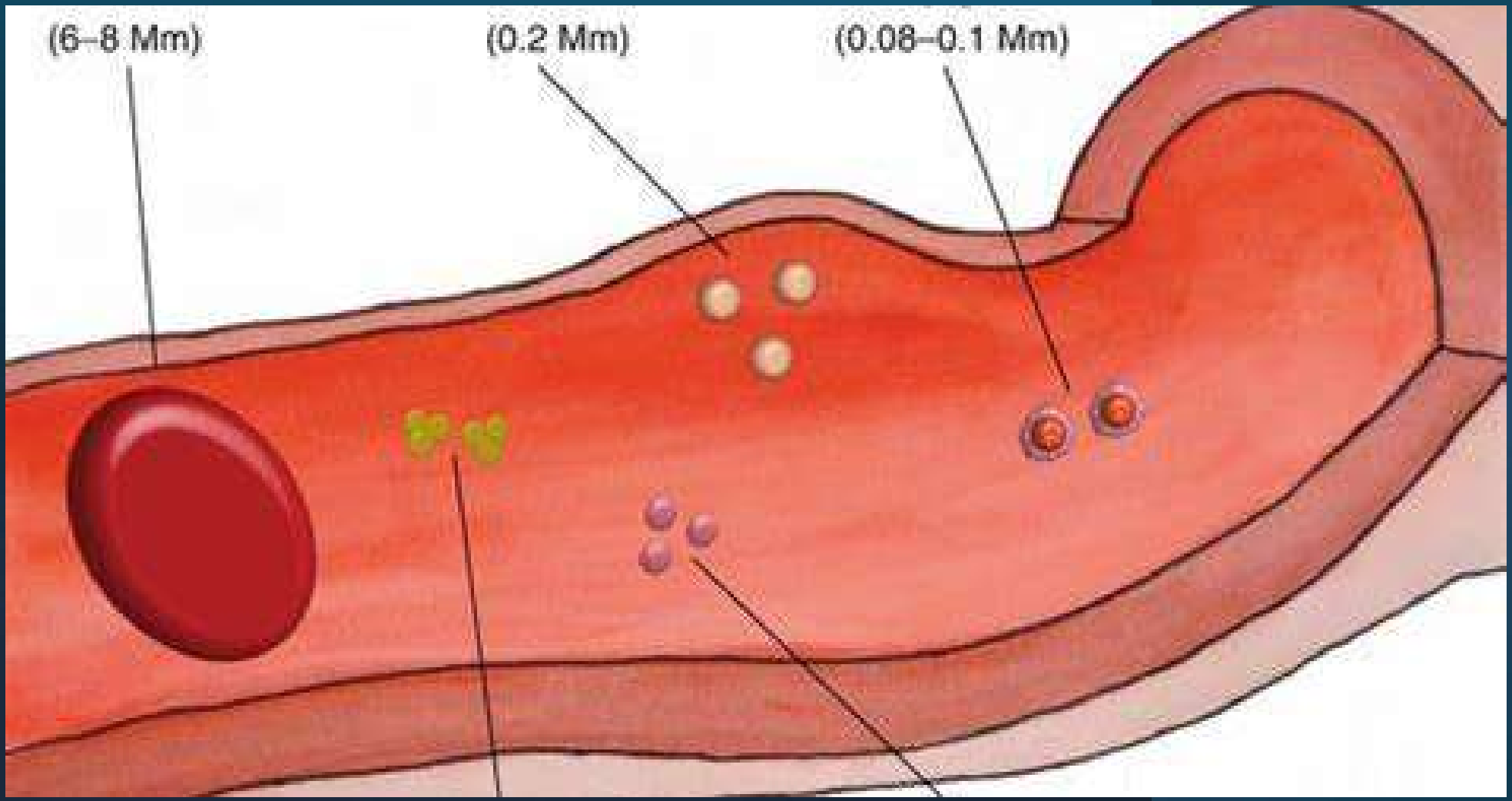
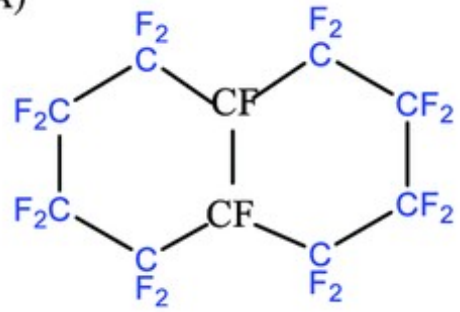



**Fig. 1: Artificial blood substitutes**

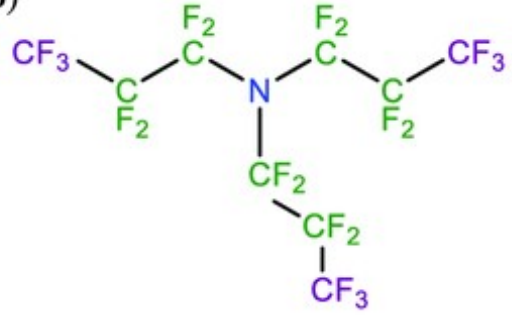

(6–8 Mm)
(0.2 Mm)
(0.08–0.1 Mm)

# Perfluorocarbons (PFC's):

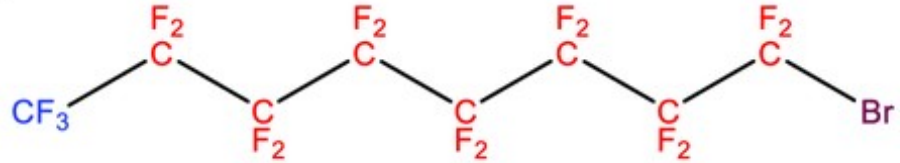
(A)



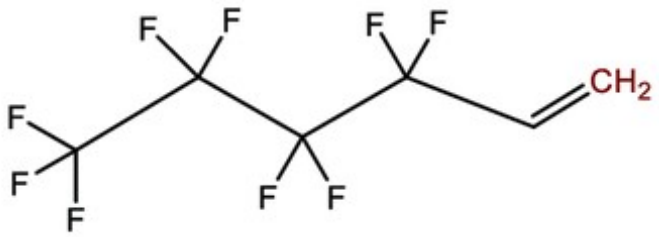

(B)




C)




(D)



# PFCs

- Used in refrigerants and fire extinguishers
- PFCs are biologically inert materials that can dissolve 50 times more oxygen than blood plasma
- Size = 0.2 micron in diameter with a phospholipid surfactant coating
- Diameter is approximately 1/40 the size of a natural RBC, allowing it to transverse capillaries where normal RBCs cannot go
- Its molecular structure consists of carbon and fluorine
- Relatively inexpensive to produce
- After production, they are added to a mixture of antibiotics, vitamins, nutrients, and salts, producing a mixture containing 80 different components, performing many of the vital functions of natural blood

# PFC’s Advantages:

- Allow for easy transportation of oxygen into tissues
- Inexpensive
- Minimize the effects of factors like Ph and temperature in blood circulation (heat resistant up to 300° C without molecular structural change)
- Helps to increase the solubility of oxygen in plasma up to 50x

# PFC's Disadvantages:

- Unable to remain mixed as aqueous solution, therefore, must be prepared as an emulsion for use
- Often causes flu-like symptoms
- Decrease blood platelet count = thrombocytopenia
- Phagocytosis of fluorocarbon emulsion by recipients' own immune system
- Realistically only delivers 0.4 mL of oxygen per 100 mL, (patient therefore require O2 supplementation; most of O2 carried by emulsion unloads before reaching microvasculature)

# Disadvantages cont..

- Excreted slowly, leaving residual metabolites within the body for months after administration
- Product stability time = 8 hours, creating difficulties for use in coronary balloon angioplasty, the indication for which the product had gained its approval (Fluosol, removed 1994)
- Oxygent: Increased stroke rates in phase 3 trials

| Product | Manufacturer | Location of Clinical Use | FDA Approval Status | Current Status |
|---|---|---|---|---|
| Flusol-DA-20 | Green Cross Corporation (Osaka, Japan) | Japan United States | Yes in 1989 | Discontinued due to side effects with limited success |
| Oxygent | Alliance Pharmaceutical Corporation (San Diego, CA) | Europe China United States | Not approved; reached phase II trials | Discontinued due to costs |
| Oxycyte | Synthetic Blood International (Costa Mesa, CA) | United States | Not approved; reached phase IIB trials | Discontinued due to lack of enrollment into phase II trials |
| Perftoran | Russian Academy of Sciences (Puschino, Russia) | Russia Mexico | Not approved | Rebranded as Vidaphor (Fluor02 Therapeutics, Inc., Boca Raton, FL) in the United States and currently awaiting clinical trials |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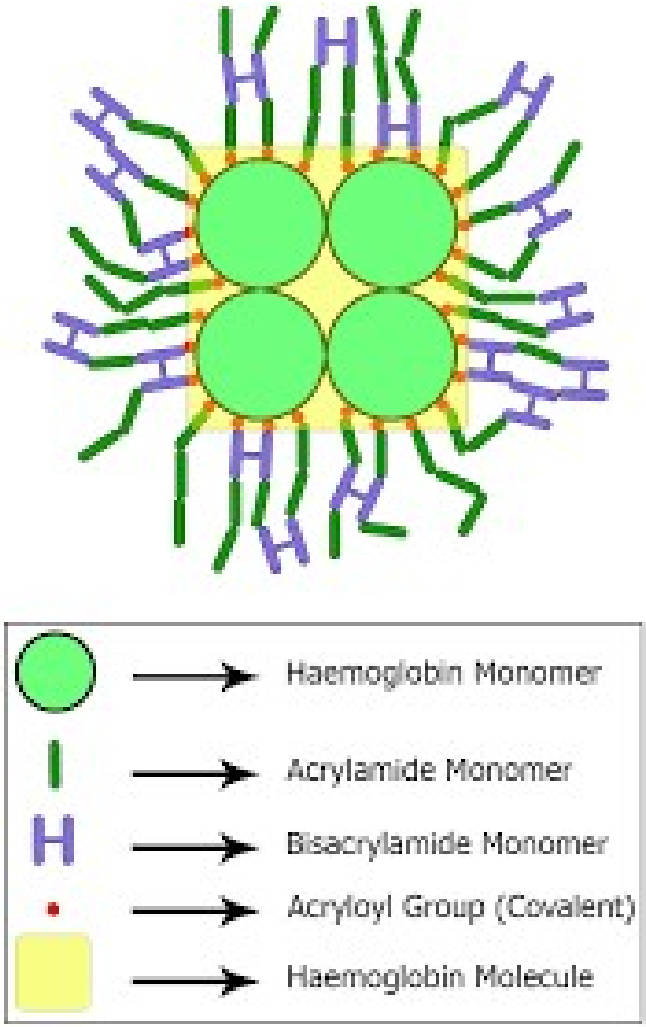
СТЕРИЛЬНО ВНУТРИВЕННО

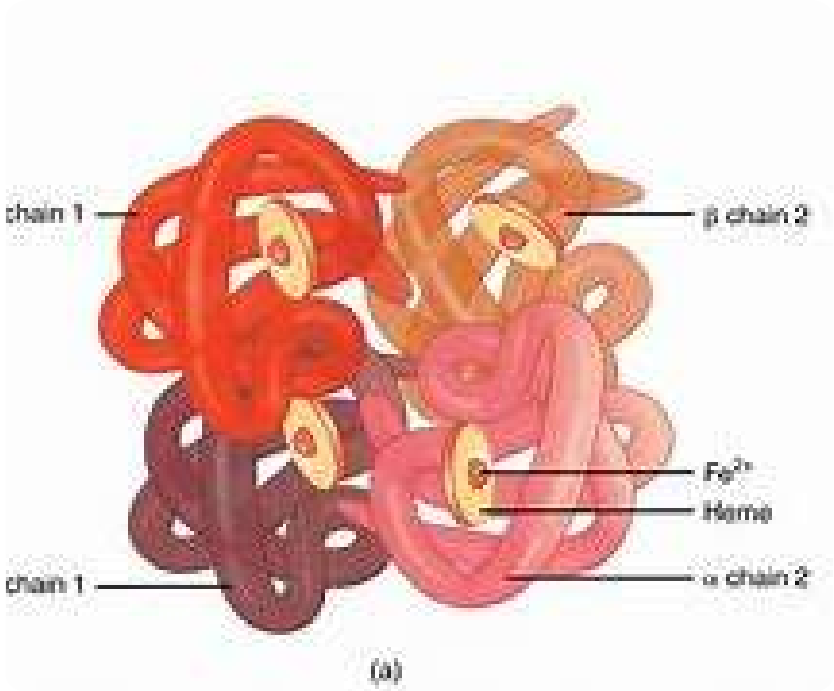
# Hemoglobin Based Oxygen Carrier

HBOCs



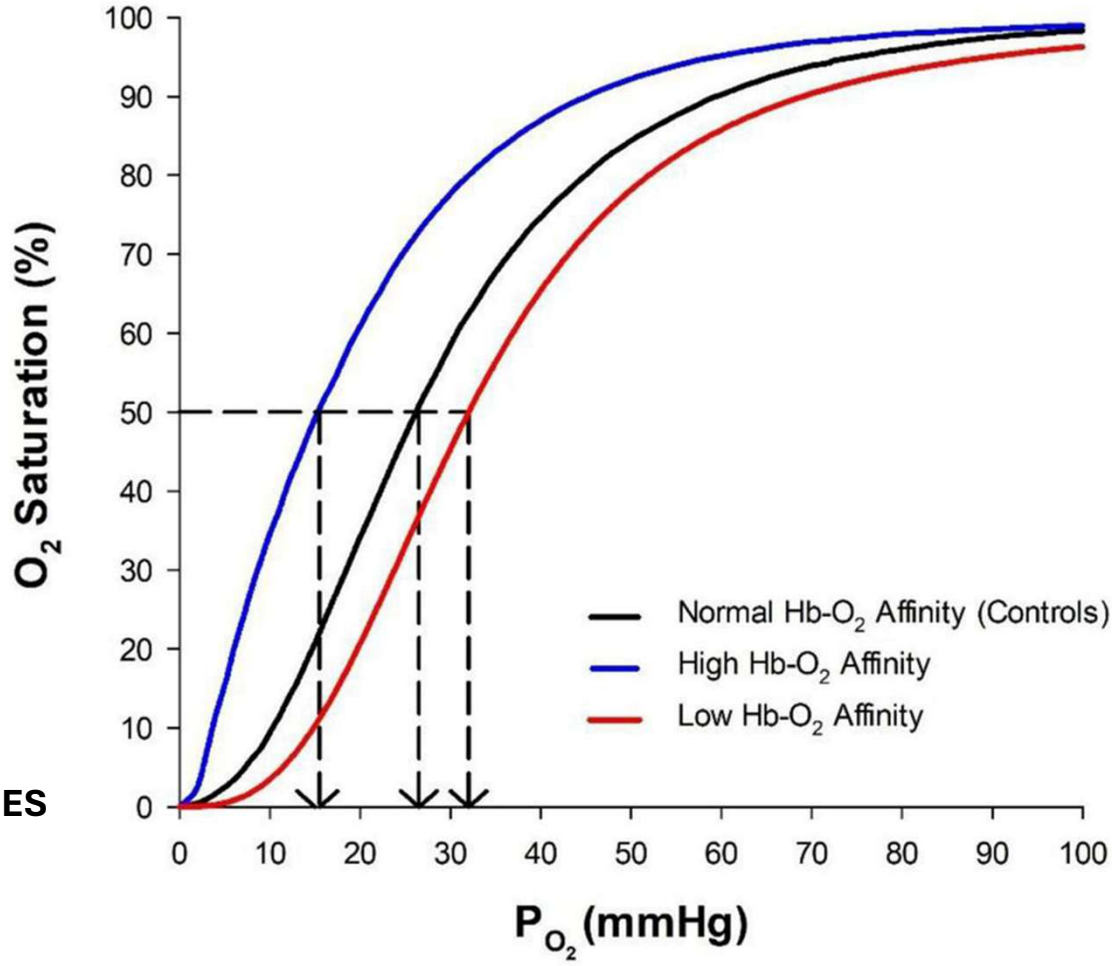
# Hemoglobin Based Oxygen Carrier

- Created as a mechanism to mimic the oxygen-carrying role of hemoglobin in the body, while reducing the need for real human hemoglobin
- Created is a Hb structure WITHOUT the red blood cell coating/membrane; “Unprotected Hemoglobin”
- HBOCs are manufactured from sterilized hemoglobin from expired human blood, cow blood, hemoglobin-producing genetically modified bacteria, or human placentas
- Through a chemical process called polymerization, 2 or more molecules bond together to form a larger HBOC molecule
- Circulate in human blood for only 1 day; natural red blood cells remaining blood stream for 100 days




- **One Hgb molecule carries 4 O2 molecules**
- **260+ million Hgb per ONE RBC cell**

LUNGS

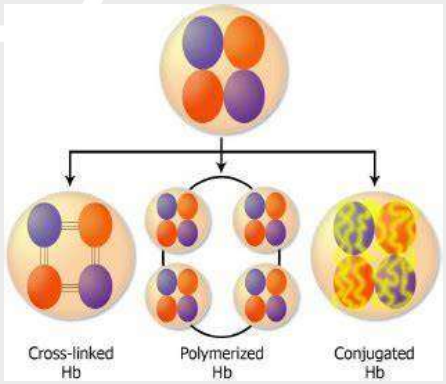


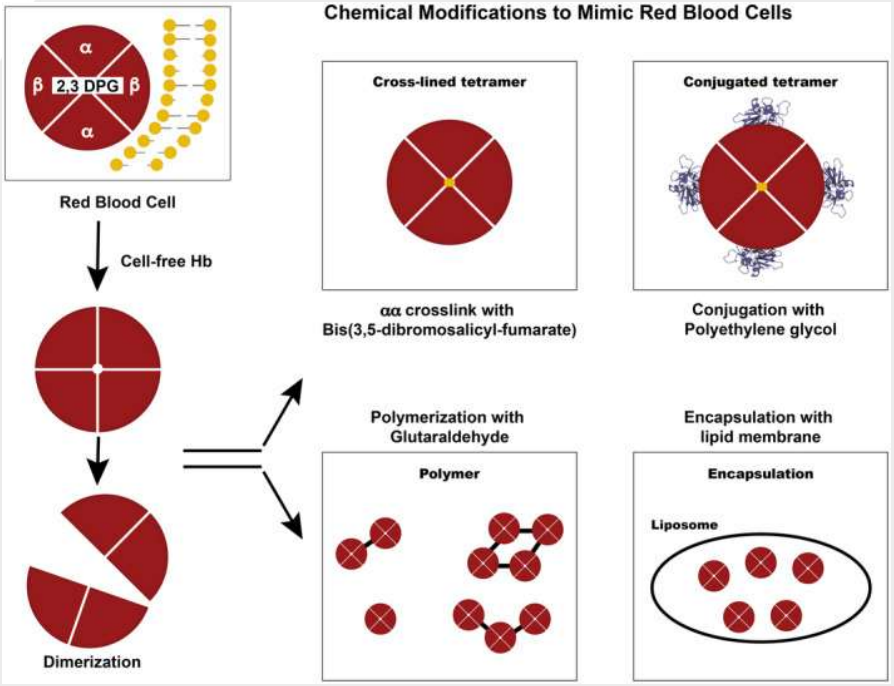

TISSUES

# Four major classes of cellular hemoglobin oxygen carriers exist

1. Cross-linked type: Man made HBOC is cross linked with a sugar molecule (diaspirin/ raffinose), creating a stable tetramer which prevents detachment; (prevent renal damage via filtration)
2. Polymerized type: Polymerization of HBOCs, which increase the size of acellular hemoglobin, minimizing extravasation and prolonging half-life within the intravascular space; (surface amino acid group linked with a reagent like glutaraldehyde).
3. Conjugated/ Surface modified: A polymer is bound to the surface of Hb, protecting it from free radicals and enlarging each molecule which significantly decreases its chances of being cleared by kidneys and penetrating into vascular walls- avoiding nitric oxide scavenging
4. Encapsulated: The HBOC is within a phospholipid bilayer capsule mimicking cellular membranes of RBCs; (Dr. Thomas Chang, 1957)

(Recently, a phase 1 clinical trial in humans was approved to Japan 11/22)


Cross-linked Hb
Polymerized Hb
Conjugated Hb


Chemical Modifications to Mimic Red Blood Cells
α
β 2,3 DPG β
α
Red Blood Cell
Cell-free Hb
Dimerization
Cross-lined tetramer
αα crosslink with
Bis(3,5-dibromosalicyl-fumarate)
Conjugated tetramer
Conjugation with
Polyethylene glycol
Polymerization with
Glutaraldehyde
Polymer
Encapsulation with
lipid membrane
Encapsulation
Liposome

# List of HBOC's

1. HemAssist- Human use first reported in 1949- went to phase III trial; adverse effects consisted of pancreatitis and myocardial infarction
2. PolyHeme- Underwent all phase I, II, III clinical trials; product failed to meet the non-inferiority goal for 30-day mortality in phase III clinical trial
3. Hemopure- Crosslinked bovine hemoglobin dissolved in salt solution; supported in South Africa in 2001 and in Russia in 2006; achieved FDA expanded approval in U.S.; (never obtained full FDA approval)
   - Was evaluated in human cardiac trials, showing increased coronary oxygenation and perfusion to distal tissue spaces; uploads oxygen in lungs and offloads oxygen in all tissues, including occluded arteries
   - Could potentially restore brain circulation and cellular function up to 4 hours after death in animal models following prolonged global ischemia (published 4/19 in *Issues of Nature*)

# Limitations to HBOCs:

- Free hemoglobin scavenge nitrous oxide, mediating vasoconstriction

  -Vasoconstriction leads to tissue hypo-oxygenation and subsequent systemic hypertension

  **(keep in mind, NO regulate endothelial mechanisms of smooth muscle relaxation- vasodilation)**
- Produces toxic free radicals, capable of inducing cell damage
- Iron in free hemoglobin can be oxidized to form Met-Hb, which has a low oxygen carrying capacity: $Fe^{2+} \rightarrow Fe^{3+}$ (ferric state)
- HBOC's have a decreased/immature offloading capacity of oxygen to tissues
- Bovine produced HBOCs increase concerns for encephalopathy/ Creutzfeldt Jacobs Dz

| TYPE OF HBOC | PRODUCT | BIOGENESIS | ACTION | PROPERTIES |
|---|---|---|---|---|
| Cross-linked HBOC | Diaspirin cross-linked Hb (DCLHb) or HemAssist | Human hemoglobin | Carrier of oxygen | In phase III clinical trial, it seems to increase mortality rates (6), lacking the ability to outregulate the oxidative state of iron in their heme group (4) |
| Polymerized HBOC | Hemopure | Glutaraldehyde bovine Hb | Carrier of oxygen | Lacking the ability to outoregulate the oxidative state of iron in their heme group (4), contains higher amount of free $\alpha 2\beta 2$, increases the risk of cardiovascular problems, risk of transmission of diseases due to the use of bovine hemoglobin (6) |
| | PolyHeme | Glutaraldehyde, pyridoxal human Hb | Carrier of oxygen | Increasing the risk of cardiovascular problems (6), trauma victims (6) |
| | Oxyglobin | Bovine hemoglobin | Carrier of oxygen | Lacking the ability to outoregulate the oxidative state of iron in their heme group (4), risk of transmission of diseases due to the use of bovine hemoglobin (6) |
| | PolyHb-SOD-CAT-CA | Bovine hemoglobin | Carrier of oxygen, removal of oxygen radical, transportation of $CO_2$ | Risk of transmission of diseases due to the use of bovine hemoglobin (6) |
| | PolyHb-Fibrinogen | | Carrier of oxygen and coagulation | Lacking the ability to outoregulate the oxidative state of iron in their heme group (4) |
| Conjugated HBOC | Hemospan | Maleimide PEG-human Hb | Carrier of oxygen | Lacking the ability to outoregulate the oxidative state of iron in their heme group (4) |
| | MP4 | Malemide PEG-hemoglobin | Carrier of oxygen | |

# Hemopure

- Considered the most successful unencapsulated HBOC to date
- Developed in 1990s
- Its Hgb extracted from cow RBCs, purified by removing pathogens, bonding four proteins together, creating a tetramer
- Early approval in South Africa 2001, where HIV crisis made blood transfusions high risk
- JAMA 2008 published a meta-analysis, which concluded all HBOC's were intrinsically toxic to the heart and that patients treated with them were 30% more likely to die than those transfused conventionally; VASOCONSTRICTION=HYPERTENSION=CADIOVASCULAR EVENT

# Hemopure

- The CEO of Biopure, believes that the blood substitute in appropriate situations, is safer than a transfusion with packed RBCs; feels that Hemopure got a “raw deal”.
- Both Jehovah witness and sickle cell patients are still being treated successfully with Hemopure; University of Pittsburgh, Medical Center;  University of Minnesota
- 2/24: Women with leukemia, had a Hgb of 2.5, was treated with 17 units of Hemopure successfully; (Hgb raised to 9.7g/dl)

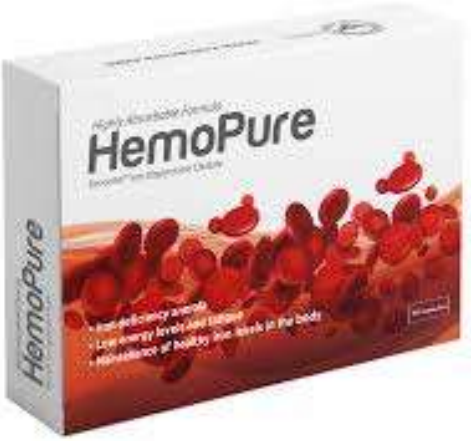
# Overall summary of side effects

- HYPERTENSION
- TACHYCARDIA
- STROKE
- HEART ATTACK
- RENAL FAILURE

ALL CAUSED BY VASOCONSTRICTION AND NAROWING OF THE BLOOD VESSELS TRIGGERED BY THE FREE HB (NITROUS OXIDE SCAVENGE)


Hemopure


HEMOPURE


HemoPure
HemoPure

# RBCs Differentiated From Stem Cells

- Red blood cell created from a stem cell
- For patients requiring chronic blood transfusions, rare blood groups, autoantibodies
- Derived from bone marrow, cord blood, embryonic stem cells, induced pluripotent stem cells
- Mechanism: Providing optimal culture conditions for cord blood-derived hematopoietic stem cells, creating a subsequent co-culture of erythroid progenitors with human fetal liver stromal cells, adding C-MYC and BCL-XL to progenitor cell
- Final maturation process creates a cell comparable to natural RBCs
- Extremely Expensive to complete- COSTS $$$$$!!

# Future Endeavors..

# ErythroMer:

- Experimental blood substitute developed by Allan Doctor, MD at the University of Maryland, School of Medicine
- Produced from recycled human hemoglobin, wrapped in a thin lipid nanoparticle membrane, which covers the free Hgb
- The membrane coat slows the nitrous oxide binding, which occurs faster with free Hgb of HBOCs, …therefor limiting NO scavenging
- ErythroMer maintains vital flow of oxygen to organs acutely, specifically for trauma and other emergency settings;  has exceptional offloading capacity of O2 to all tissues
- Freeze dried powder that remains unstable for years, reconstituted with saline
- Used for any individual blood type because its membrane has no RBC surface antigen/protein

| Red blood cells | ErythroMer |
| --- | --- |
| SHELF LIFE | SHELF LIFE |
| 42 days | 2 years |
| SIZE | SIZE |
| 7–8 µm | ~0.2 µm |
| COMPATIBILITY | COMPATIBILITY |
| By blood type | Universal |

A. FISHER/*SCIENCE*

# Clinical Trial Findings:

- Latest preclinical data demonstrated effective oxygen delivery in animals that had 70% of their blood volume replaced with ErythroMer.
- In rabbits, after ½ of their blood volume is removed, infused fluid containing ErythroMer, the animals' vitals were resuscitated to normal levels, as if real blood had been transfused
- Phase 1 trial in humans were cut short due to coronavirus pandemic in 2020
- Initial results revealed minimal temporary side effects included rash and fever

# Where are we today?...

- For now, no human blood substitute is commercially available in the USA
- In 2023, Defense Advanced Research Project Agency (DARPA) announced a $46 million dollar grant to UMD-led consortium to develop a shelf stable, field deployable, whole blood substitute with ErythroMer.
- DARPA project also aims to develop synthetic platelets and freeze-dried plasma

# Questions?

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# Outreach

- Physician available 24/7
  - Practitioners with transfusion-related questions/issues
  - Blood bank-related questions/issues
  - (515) 309-4840
- Educate the medical community to keep them up to date on transfusion-related topics
  - Presentations to medical personnel
  - Contact me: nicole.saviano@lifeservebloodcenter.org
- Quarterly webinars
  - *https://www.lifeservebloodcenter.org/for-hospitals/resource-guide/education*

# Thank you!

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