Artificial Blood

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• The blood of vertebrates is the most complicated fluid to be found in the world of living organisms. Compounded of a dozen essential ingredients, sustaining a multiplicity of activities, the fluid pathway for a variety of chemical and hormonal integrations of function, the source of food and oxygen for every tissue, it defies laboratory synthesis. At the very beginning we must recognize that there is no complete substitute for blood. Yet biologists and physiologists, no less than clinicians, are so frequently confronted with situations where normal blood cannot be obtained, or where the problem at issue can only be solved by a simplification of conditions, that a substitute for blood has become one of the most pressing needs of the experimental laboratory.

(Amberson, 1937)
Artificial Blood

• A man made substance that can be transfused into an individuals body and perform the tasks of blood
The Blood

- Human life Depends on Blood
- Connective tissue
- Plays crucial role in supply defence and communication in the body
- fluid pathway for a variety of chemical and hormonal integrations of function
Blood con`t

- The blood is made up of four distinct parts:
  - Red blood cells
  - White blood cells
  - Plasma
  - Platelets
Red Blood Cells

- Contain Hemoglobin
- Carries oxygen to cells and simultaneously carry carbon dioxide away from the cells
- Form from stem cells
- Identical
- Contain no nucleus
White Blood Cells

- Fight disease
- Many different types of cells:
  - Phagocytic
  - Lymphacytic
  - T-cells
Plasma

- Matrix that carries blood cells through circulatory system
- ~ 92% water
- Other ~ 8% is protein and electrolytes which perform many essential tasks in our blood and body
Platelets

- Tiny cell fragments
- Essential in blood clotting
- If we did not have these cells we would rapidly bleed to death even with the smallest cut
Fluid Mechanics of Blood

• Density = $1060 \text{ kg}\cdot\text{m}^{-3} @ 30^\circ\text{C}$
• Viscosity of blood = $0.0027 \text{ N-s/m}^2 @ 37^\circ\text{C}$
• Flow of blood can be found by Hagen-Poiseuille’s equation, which defines the flow $Q$ in a tube as:

$$Q = \pi R^4 \left(8 \eta^{-1} \frac{\Delta P}{\Delta L}\right)$$

$R$ is the tube radius, $\eta$ is the fluid viscosity, and $\frac{\Delta P}{\Delta L}$ is the local longitudinal pressure gradient.
Brief History

Brief overview of the history of blood transfusion and the search for blood substitutes
History of Transfusions

- Circulation described and published, 1616 and 1628
- First successful transfusion from animal to animal, 1665
- First human transfusions, resulting in death, 1667
- Transfusions again accepted as relatively safe (Blundell’s research), 1818
- Plasma and serum described, 1871
- RBC antigens (blood types) described by Landsteiner, 1901

Figure 1.1 Blood transfusion from animal to man in 1672 (from Kilduffe and DeBakey, 1943, with permission).
History of Substitutes

- Gum-saline and wine tested as substitutes, 1863
- Milk tested during cholera epidemic, 1878
- Hemoglobin transfusions in humans, 1916
- Albumin-Hemoglobin mixtures, ~1941-45
- Encapsulated hemoglobin, 1957
- PFC exchange, ‘Bloodless’ mouse, 1966-8
- Polymerization of hemoglobin and polyhemoglobin, 1973, 76
- Human trials with modified hemoglobin, 1989

Figure 1.2 Liquid-breathing mouse. The mouse is totally immersed in perfluorocarbon (FC-80, butyltetrahydrofuran) which has been saturated with oxygen by bubbling at room temperature. Such a mouse can survive liquid breathing for many hours (From Clark, 1985).
Current Transfusion Technology

Overview of current transfusion treatments and associated problems
Plasma

- Protein rich liquid in blood which helps to circulate blood components, aid in immune response and clotting.
- Can be administered to treat:
  - Some bleeding disorders (blood volume increaser)
  - Liver diseases
  - Shock, Burns
  - Cancer and Bone marrow therapy
  - Operations
Red Blood Cells

- Carry oxygen to tissue and responsible for CO$_2$ removal in lungs
- Donated blood undergoes leukoreduction, whereby white blood cells are removed.
- Can be administered to treat:
  - Accidents (trauma)
  - Surgical operations
  - Anaemia
Platelets

• ¼ the size of RBCs, cell fragments required to produce clotting, produced by megakaryocytes in bone marrow.

• Can be administered to treat:
  – Leukemia
  – Cancer Patients
  – Prologned Bleeding
Saline Solution

- Salt water, (normal saline 0.9% weight by volume)
- Can be administered to treat:
  - Dehydration
  - Low blood pressure (as a volume increaser)
Problems

• Shelf life:
  – RBCs: 42 Days @ 2-6°C
  – Platelets: 5 Days @ 20-24°C (under agitation)
  – Fresh Frozen Plasma: One Year @ -30°C
  – Cryoprecipitate: One Year @ -30°C

• Specific storage requirements
  – Not really conducive to emergency situations
## Blood Types

<table>
<thead>
<tr>
<th>Blood Type (Donor)</th>
<th>% of Blood Type Amongst all Canadians</th>
<th>Patient Types Compatible with the Red Blood Cells of Donor</th>
<th>Patient Types Compatible with the Plasma of Donor (Rh not indicated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+</td>
<td>36</td>
<td>A+, AB+</td>
<td>A, O</td>
</tr>
<tr>
<td>A-</td>
<td>6</td>
<td>A-, A+, AB-, AB+</td>
<td>A, O</td>
</tr>
<tr>
<td>O+</td>
<td>39</td>
<td>O+, A+, B+, AB+</td>
<td>O</td>
</tr>
<tr>
<td>O-</td>
<td>7</td>
<td>All Blood Types</td>
<td>O</td>
</tr>
<tr>
<td>B+</td>
<td>7.6</td>
<td>B+, AB+</td>
<td>B, O</td>
</tr>
<tr>
<td>B-</td>
<td>1.4</td>
<td>B-, B+, AB-, AB+</td>
<td>B, O</td>
</tr>
<tr>
<td>AB+</td>
<td>2.5</td>
<td>AB+</td>
<td>All Blood Types</td>
</tr>
<tr>
<td>AB-</td>
<td>0.5</td>
<td>AB-, AB+</td>
<td>All Blood Types</td>
</tr>
</tbody>
</table>
Supply and Demand

- 80 million units of blood collected each year worldwide
  - 38% collected in developing countries, but 82% of the world’s population lives there.
- Donation use increasing rapidly, but donations remain mostly stagnant.
- Extremely high demand for O- blood type, but only 7% of population can donate it.

In 2007/2008, Canadian Blood Services experienced a 1.1 per cent drop in the number of active donors year over year and a 0.3 per cent drop in whole blood donations. The forecast over the next three years suggests that whole blood collections must increase two per cent a year to meet a similar increase in hospital demand.
Supply and Demand

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Order fill rates of red blood cells

Order fill rate refers to the percentage of hospital orders we are able to meet. In 2007/2008 with the exception of platelets, we experienced lower than expected fill rates in large part due to collections and inventory issues experienced last year.
Infectious diseases

• Before 1990, too few or simply no tests for Hepatitis-C in Canada, many believed to have contracted via transfusions.
• As many as 1000 people expected to have contracted HIV during a similar crisis in Canada via an anti-clotting solution between 1980 and 1990.
• Screening methods now in place:
  – Since 1985, HIV and Hep-C screening in place
  – Nucleic acid testing
• However, more concerns are being discovered:
  – Prions
Introduction to Artificial Blood
Introduction to Artificial Blood

• artificial blood or blood substitutes is either used to fill fluid volume and/or carry oxygen and other gases in the cardiovascular system

• The oxygen carriers which most resemble blood fall under 2 groups:
  - perfluorocarbon based
  - hemoglobin based
Volume Filler

- Used when blood loss occurs
- Provided blood volume is maintained by volume Filler, a resting patient can safely tolerate very low hemoglobin levels, less than 1/3rd of a healthy person
- Ex. Saline
Oxygen Carriers

- Performs the most important task of blood
- Beneficial replacement of blood:
  - in war zones
  - natural disasters
  - on route to hospital
  - religious groups
Oxygen Carriers Cont.

- Benefits over real blood:
  - longer shelf life
  - no blood typing required
  - easily transportable
  - no disease
Oxygen Carriers Cont.

• Artificial blood takes advantage of passive diffusion, in which oxygen moves from an area of high concentration (the blood) to an area of low concentration (the tissues/cells).
Perfluorocarbon Based RBC Substitutes
What are they?

- Linear or cyclic carbon chain which is highly substituted by fluorine atoms.
- Initially developed as chemically inert materials intended for use in handling unstable radioisotopes.
- Also used as non-stick coating for fabrics and cookware.
Physical Properties

- Chemically inert (C-F bond among strongest covalent single bond)
- Liquid at room temperature
- Very small, molecular scale.
- Highly soluble for respiratory gases (O, N, CO₂)
  - Can dissolve $3 - 6 \times 10^{-4}$ml/mmHg of O₂ per gram.
  - 20-30 times the solubility of O₂ in plasma or water.
  - Can dissolve 40-70% oxygen per unit by volume, blood only ~20%.
- Not miscible in water or plasma
  - Possibly the most hydrophobic substance ever invented.
  - Must be emulsified with a separate agent to allow stable solution in blood plasma.
Physical Properties

• Amount of oxygen gas which can be dissolved is directly proportional to the partial pressure of oxygen in solution.
  – At high enough $P(O_2)$, should be able to dissolve as much oxygen as blood.
  – Gasses are dissolved passively through diffusion. High concentrations of oxygen in lungs to low concentration in PFCs, and then from high concentrations in PFCs to low concentrations in cells. Hemoglobin delivers via binding oxygen.
  – Average arterial $P(O_2)$ is $\sim$100mmHg
Why is oxygen content so low?

![Graph showing oxygen content vs. PO2 for blood, PFC Emulsion, and Plasma](image)

**Figure 13.4.** Oxygen-carrying capacity of whole blood, a perfluorocarbon emulsion (Oxygent) (70), and plasma, as a function of PO2. The data for whole blood and plasma are from healthy humans at a hemoglobin concentration 15 g/dl (71). inset: the same at higher PO2. (Modified from Stowell CP, Levin J, Spieks BD, et al. Progress in the development of RBC substitutes. Transfusion 2001;41:287–299, with permission.)
PFC Transfusions

• Due to immiscibility, PFC must be emulsified before it can be transfused.
  – Albumin, fats, or lecithin (egg-yolk phospholipid)
• Emulsification decreases the oxygen carrying capacity.
• As a result, patients require supplemental oxygen when being administered PFCs.
• Emulsification increases particle size: 0.1-0.2μm desired
  – Increases viscosity of solution
  – Larger particles are removed from blood more quickly
  – Larger particles decrease O₂ capacity further
PFC mechanism

- Injected into blood in emulsifying agent.
- Oxygen diffuses across alveoli into PFCs.
- PFCs deliver oxygen to cells, take up CO$_2$.
- Repeat until emulsifying agent breaks down in circulation.
- Emulsifying agent removed by macrophages, kidneys, or liver. PFCs exhaled from lungs. (4 – 12 Hour half lives)
  - After Emulsifier digested, PFCs can reside in tissues at low concentrations until they filter to the blood.
Advantages of PFCs

- Production does not depend on blood, very easy to manufacture.
- Chemically inert, very biocompatible.
- No antigens, universal donor.
- No pathogens or infectious agents.
- Storage time: depends on specific product, generally 1-2 years at room temperature or below.
Problems with PFCs

• Emulsification is difficult; proper sized particles, biocompatible agent, retention in tissues.
  – Too small and vapour pressure is too high, oxygen doesn’t cross alveoli. Too large and particles are filtered out of blood quickly.
  – PFCs can take weeks to filter out of tissues. Possibility of accumulation and circulatory blockage.
  – Flu like symptoms when digesting emulsifying agent (macrophages).
  – Decrease in platelet count related to digestion and filtration of emulsifier. However, function of platelets unaffected and clotting appeared normal after testing.
Problems with PFCs

Figure 24.9 Particle size growth over time in fluorocarbon emulsions is due to molecular diffusion (a) rather than to droplet coalescence (b). The thin arrows in scheme (a) represent individual molecules leaving the smaller droplets to join larger ones, where the chemical potential is lower.
Problems with PFCs

• Requirement of supplemental oxygen.
  – Some PFC products require almost atmospheric \( P(O_2) \) to deliver acceptable amounts of oxygen.
  – Supplemental oxygen may not be readily available in emergency situations, (I.e. battlefield, site of disaster) or in developing nations.
  – Possibility of free radicals and oxygen toxicity when high concentration oxygen gas is used.
Problems with PFCs

• Vasoconstriction due to lower viscosity:
  – Viscosity is proportional to pressure when flow remains constant.
  – Initially thought that low viscosity would improve hemodynamics.
  – A decrease in viscosity without an increase in flow lowers pressure. At microcirculatory level, this decreases shear stresses on vascular endothelial cells, which respond by restraining their relaxing factors, causing constriction. Part of the bodies way of maintaining blood pressure.
  – Larger particles increase viscosity, but are very hard to stabilize in blood.
Current PFC projects

• **Oxygent (Alliance Pharmaceuticals)**
  - Phase III clinical trials (halted due to strokes).
  - One unit of Oxygent contains 1-2 units of blood worth of oxygen at 60g PFC per 100ml.
  - Approximate viscosity of blood, ~4-5cP. No vasoactivity and no affect on cardiac output.
  - 24 month shelf life at 2-8°C, ‘several weeks’ at room temp.
  - Half life of 12 hours in blood.
Current PFC Projects

Figure 24.3 Oxygen solubility in fluorocarbons follows Henry’s law – i.e., it is directly proportional to the gas’ partial pressure, as expected in the absence of chemical bonding, while oxygen uptake by hemoglobin, which binds oxygen through a strong covalent bond to its iron atoms, follows a sigmoid curve that saturates when the partial pressure of oxygen in earth’s atmosphere is attained. Oxygen extraction from a PFC emulsion can reach 90 per cent of oxygen content.
Current PFC Projects

**Augmented-ANH with Oxygen**

Figure 24.3 Oxygen solubility in fluorocarbons follows Henry’s law – i.e., it is directly proportional to the gas’ partial pressure, as expected in the absence of chemical bonding, while oxygen uptake by hemoglobin, which binds oxygen through a strong covalent bond to its iron atoms, follows a sigmoid curve that saturates when the partial pressure of oxygen in earth’s atmosphere is attained. Oxygen extraction from a PFC emulsion can reach 90 per cent of oxygen content.
Hemoglobin Based

Crosslinked  Polymer-conj’ed.Hb  Polym’d. Hb
Hemoglobin Based

- Often referred to as HBOC’s
- In the case of HBOC’s, oxygen is bound to the carrier hemoglobin which in turn governs the release of oxygen to the
HBOC’s

• Three Main sources of Hemoglobin:
  - Outdated red blood cells (Human)
  - Bovine
  - Recombinant (Human)
HBOC’s

**TABLE 13.4. COMPARISON OF HEMOGLOBIN SOURCES**

<table>
<thead>
<tr>
<th>Property</th>
<th>Human</th>
<th>Bovine</th>
<th>Recombinant (Human)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity</td>
<td>Limited(^a)</td>
<td>Abundant</td>
<td>Moderate</td>
</tr>
<tr>
<td>(P_{50})^b</td>
<td>(\sim)</td>
<td>(\leftrightarrow)</td>
<td>(\leftrightarrow)</td>
</tr>
<tr>
<td>Immunogenicity(^c)</td>
<td>Very low</td>
<td>Low</td>
<td>Very low</td>
</tr>
<tr>
<td>Infection risk</td>
<td>Very low</td>
<td>Very low</td>
<td>Very low</td>
</tr>
<tr>
<td></td>
<td>? New pathogen</td>
<td>? Bovine pathogens</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Outdated red blood cells.
\(^b\) Unmodified hemoglobin.
\(^c\) May depend on modification procedures.
\(\downarrow\), decreased; \(\leftrightarrow\), unchanged.
### TABLE 13.2. POTENTIAL ADVANTAGES AND DISADVANTAGES OF UNMODIFIED HEMOGLOBIN

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>High capacity for oxygen and carbon dioxide</td>
<td>Rapid clearance</td>
</tr>
<tr>
<td>Functions at physiologic PO$_2$</td>
<td>Renal toxicity</td>
</tr>
<tr>
<td>Low viscosity (?)</td>
<td>Vasoactivity</td>
</tr>
<tr>
<td>High oncotic pressure</td>
<td>Increased oxygen affinity</td>
</tr>
<tr>
<td>Absence of red blood cell antigens</td>
<td>Autooxidation</td>
</tr>
<tr>
<td>Prolonged shelf life</td>
<td>Immunogenicity (modified or nonhuman)</td>
</tr>
<tr>
<td>Purification and viral inactivation possible</td>
<td>Potentiation of sepsis (?)</td>
</tr>
</tbody>
</table>
 Modifications to Hemoglobin to Combat these disadvantages

- Rapid Clearance, and Renal toxicity due to rapid dissociation of hemoglobin also increased oxygen affinity.
Modifications to Hemoglobin to Combat these disadvantages

• Autoxidation can cause adverse effect with cell signalling

Solution:

• direct modification(s) of the hemoglobin molecule; including chemical and/or genetic engineering of the protein; cross linking red blood cell antioxidant enzymes to the HBOC

• indirect use of reducing agents or antioxidants
Modifications to Hemoglobin to Combat these Disadvantages

- Autoxidation can cause adverse effects with cell signaling

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- Direct modification(s) of the hemoglobin molecule; including chemical and/or genetic engineering of the protein; cross-linking red blood cell antioxidant enzymes to the HBOC
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![Chemical Reaction Diagram]

HbFe$^{3+}$ + Ascorbic acid (AA) $\rightarrow$ Ascorbic acid radical (AAR) $\rightarrow$ Dehydroascorbic acid (DHA) $\rightarrow$ + HbFe$^{2+}$

NADH Oxido-reductase

Glut-1

GSH

Glutathione reductase

AA

AAR Reductase

AAR

NADH

NAD$^+$

DHA

H$_2$O$_2$
Modifications to Hemoglobin to Combat these disadvantages

- Vasoconstriction one of the main failures of HBOC’s and artificial blood all together saw the failure of many HBOC’s ex. Caused massive Heart attacks in 30% of patient given HBOC’s

Solution:
- no real solution yet but promise has been shown with the production of larger molecules that interact less with vessel walls
Examples of HBOC’s

• Hemopure  
  - in use in South Africa  
  - viscosity=1.3 @37°C  
  - Storage temp 3-20°C  
  - Bovine Hemoglobin
Examples of HBOC’s

- Hemospan
  - from human hemoglobin
  - effective in low concentrations
  - only allowed 1L a patient or problems may ensue
  - still in clinical trials
Comparison

- **PFC’s:**
  - Synthetic, does not rely on natural organic sources. (unlimited production capabilities)
  - Inexpensive
  - Storage 1-2 yrs @ 2-8°C
  - Requires emulsification
  - Require supplementary oxygen (works by diffusion)

- **HBOCs**
  - Natural, relies on Hemoglobin from humans or animals.
  - Inexpensive if bovine Hemoglobin used (limited source)
  - Storage 1-3 yrs @ 3-20°C
  - Requires large amount of modification
  - No supplementary oxygen required (binds oxygen straight from lungs)
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Common Problems

- Vasoconstriction
- Particle breakdown, which causes:
  - Renal toxicity (emulsifier and hemoglobin)
  - Tissue retention (PFCs)
- Not approved for use in many countries.
Future Developments

• Use of enzymes to remove A and B antigens from RBCs to increase supply of O type blood.
• Dendrimers: Water soluble, highly fluorinated polymers, already used for drug delivery.
• Use of PFC/HBOCs to treat cells RBCs cannot reach, e.g. brain swelling/strokes.
• Use of targeted PFCs to administer lethal oxygen doses to cancer cells where operations are difficult.
Questions?
Reference Material

Reference Material