

From Artificial Red Blood Cells, Oxygen Carriers, Oxygen Therapeutics to Artificial Cells and Nanomedicine and beyond.

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Abstract

The first experimental artificial red blood cells have all 3 major functions of red blood cells (rbc). However, the first practical one is a simple polyhemoglobin (PolyHb) that only has oxygen carrying function. This is now in routine clinical use in South Africa and Russia. An oxygen carrier with antioxidant functions, PolyHb-catalase-superoxide dismutase, can fulfill 2 of the 3 functions of rbc. Even more complete is one with all 3 functions of rbc in the form of PolyHb-catalase-superoxide dismutase-carbonic anhydrase. The most advance ones are nanodimension artificial rbc with either PEG-lipid membrane or PEG-PLA polymer membrane. Extensions into oxygen therapeutics include a PolyHb-tyrosinase that suppresses the growth of melanoma in a mice model. Another is a PolyHb-fibrinogen that is an oxygen carrier with platelet-like function. Research has now extended well beyond the original research on artificial rbc into many areas of artificial cells. These include nanoparticles, nanotubule, lipid vesicles, liposomes, polymer tethered lipid vesicles, polymersome, microcapsules, bioencapsulation, nanocapsules, macroencapsulation, synthetic cells and others. These are being used in nanotechnology, nanomedicine, regenerative medicine, enzyme/gene therapy, cell/ stem cell therapy, biotechnology, drug delivery, hemoperfusion, nanosensors and even by some groups in agriculture, industry, aquatic culture, nanocomputers and nanorobotics.

Artificial Red Blood Cells

The first artificial red blood cells fulfilled the following 3 major functions of red blood cells: (1) oxygen transport (Chang, 1957) (2) carbon dioxide transport (Chang, 1964, Science) and (3) antioxidant functions (Chang and Poznanski,

Nature 1968). However, serious interest in this area did not start until the HIV contaminated donor blood crisis. By then, there was no time to carry out the much needed basic research.

Hemoglobin Based Oxygen Carriers (HBOC)

The urgency led to the development of different hemoglobin based oxygen carriers (HBOC) that has only one of the functions of red blood cells. One of these is based on the basic research on glutaraldehyde crosslinked polyhemoglobin (Chang 1971). The group from Northfield has independently developed glutaraldehyde crosslinked human polyhemoglobin and carried out very extensive clinical trials (Moore et al Gould, 2009). The group from Biopure has independently developed glutaraldehyde crosslinked bovine polyhemoglobin and has also carried out very extensive clinical trials (Jahr et al 2008). South Africa has approved the routine clinical uses of polyhemoglobin for a number of years and Russia has recently approved the routine clinical uses of polyhemoglobin. Another HBOC in the form of conjugated hemoglobin can be formed by the basic principle of crosslinking hemoglobin to polymer (Chang 1964,1972). The one in development is a PEG conjugated hemoglobins (Liu and Xiu, 2008; Winslow,2006)

HBOC only has one of the 3 major functions of red blood cells. However, the risk/benefit ratios of polyhemoglobin have already been shown in situation where rbc is not available (Moore et al 2009; Jahr et al, 2008). Furthermore, polyhemoglobin can be sterilized to be free from HIV or other infective agents and it can also be stored at room temperature for more than 1 year. This is compared to donor red blood cells that require storage at 4°C for up to only 42 days. Furthermore, there are questions of whether this length of storage of donor blood would lead to adverse effects (Roa et al 2005).

Oxygen Carriers with antioxidant functions

In the meantime, studies are being carried out towards oxygen carriers with antioxidant functions to fulfill 2 of the 3 functions of red blood cells(D'Agnillo and Chang 1998, Powanda and Chang 2002). This is important for conditions with potential for ischemia-reperfusion injury (Alayash et al 2007). One approach is in the form of PolyHb-catalase-superoxide dismutase (*DAgnillo & Chang, 1998*) that is effective in preventing ischemia-reperfusion injury in a hemorrhagic shock-stroke rat model (Powanda and Chang 2002). Hsia's group has extended this using hemoglobin with synthetic antioxidant enzymes (Buehler et al 2004)). There is also important recent research to resolve the effect of HBOC on nitric oxide in those conditions with endothelial dysfunction (Yu et al, 2010).

A blood substitute with enhanced antioxidant functions that can transport both oxygen and carbon dioxide

Sims et al (2001) carried out studies in animal using tissue CO_2 microelectrodes. They show that tissue CO_2 is not reflected by blood PCO_2 . Furthermore, tissue CO_2 increases with severity of hemorrhagic shock and is correlated with survival. We have therefore carried out research on Polyhemoglobin-catalase-superoxide dismutase-carbonic anhydrase (Bian, Rong & Chang 2011). This fulfills all 3 major functions of red blood cells in acting as O_2 & CO_2 carrier with enhanced antioxidant properties

Complete nanodimension artificial red blood cells

The original complete polymer membrane artificial red blood cells (Chang, 1964, 1972) had very short circulation time. Study was carried out to prepare artificial cells with lipid membrane in the form of lipid-protein membrane and lipid-polymer membrane [Chang 1972]. Djordjevich and Miller (1980) prepared submicron 0.2 micron diameters artificial RBC using lipid membrane vesicles to encapsulate Hb. This increased the circulation time significantly. Philips et al (1992) markedly improve the circulation time by incorporate polyethylene-glycol (PEG) into the lipid membrane. The submicron haemoglobin lipid vesicle haemoglobin approach is being extensively developed by a group in Japan towards clinical trial [Tsuchida et al 2006]. Lipid vesicles would be useful for conditions that do not require large volume of blood substitutes. Since the smaller the diameter the larger would be the surface to volume relationship, these 200 nanometer lipid vesicles have a total surface area and therefore lipid that is about 10 times that of 7 micron red blood cells. Large amount of lipid can cause the saturation of the reticuloendothelial system. We are therefore use biodegradable polymer membranes to form complete nano dimension artificial red blood cells [Chang et al 2003]. These nano artificial RBCs of 80 to 150 nanometres contain all the red blood cell enzymes. Using a polyethylene-glycol-poly lactide copolymer membrane we are able to increase the circulation time of these nano artificial red blood cells to double that of PolyHb [Chang et al 2003]. Further studies in rats by Liu and Chang (2008) show that one infusion with 1/3 the total blood volume, did not result in any adverse effects or changes in the histology or blood biochemistries when followed on days 1, 7 and 21 after infusion.

Oxygen Therapeutics

Oxygen carrier with enhanced antioxidants is not only for rbc replacement. It can be prepared with much higher antioxidant activities so that it can be used as oxygen therapeutic in conditions of severe ischemia-reperfusion as in severe sustained hemorrhagic shock, myocardial infarction, stroke or organ transplantation. Oxygen carrier with NO transport is another example of oxygen therapeutic. *Another example is oxygen carrier with platelet activity (Wong and Chang, 2007) In high blood volume loss, replacement with large volume of oxygen carrier or red blood cell alone would not replace platelets and coagulation factors. We have prepared a polyhemoglobin-fibrinogen that is effective as an oxygen carrier with platelet-like properties in rats with 98% volume exchange (Wong and Chang 2007). Oxygen carrier with anti-tumour activity in the form of PolyHb-tyrosinase has the combined function of increasing oxygen tension to sensitize the melanoma to therapy and lowering systemic tyrosine to retard the growth of this fatal skin cancer (Yu and Chang 2004).*

Beyond oxygen carrier, blood substitutes, oxygen therapeutics to Artificial Cells with its extensive therapeutic uses.

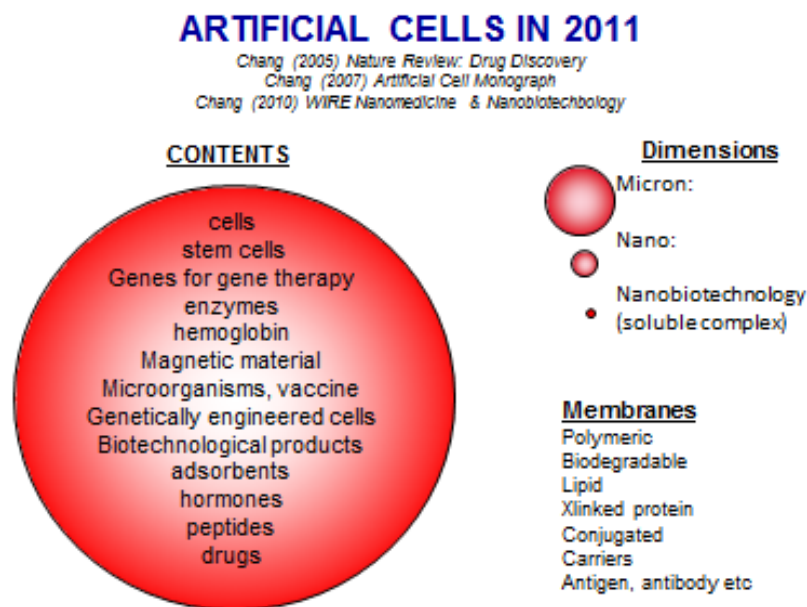


Figure 1: Possible variations in dimensions, membrane materials and contents of artificial cells (From Chang 2007, With permission from World Science Publisher)

The general principle of artificial cells can form the basis of a large number of artificial systems (Chang, 2005,2007)(Fig. 1). In addition to being of cellular dimensions in the micron range, they can also be in the macro range, in the nano range or in the molecular range. Furthermore, the membrane material includes polymer, biodegradable polymer, lipid, crosslinked protein, lipid-polymer complex, lipid-protein complex and membrane with transport carriers. The artificial cells can contain an unlimited variety of material individually or in combinations (Fig. 1). These include cells, stem cells, enzymes, multienzyme systems, hemoglobin, magnetic materials, microorganism, vaccines, gene for gene therapy, genetically engineered cells, adsorbents, drugs, hormones, peptides, proteins and others. There have been increasing and explosive interest and research activities around the world on artificial cells, especially in fields related to biotechnology, nanomedicine, nanoscience, bioencapsulation, cell therapy, blood substitutes, advance drug delivery systems and even nanoscale robotics and others (Chang, 2007). Some of these “artificial cells,” are disguised under other terminologies such as liposomes, polymersomes, nanoparticles, microcapsules, blood substitutes, bioencapsulation and so on

World-wide poll and artificial cells

In a recent world-wide poll by McGill University, the inventor of artificial cells was voted the Greatest McGillian in the University's 190 year history (www.artcell.mcgill.ca). This has less to do with the individual but more related to the potential of artificial cells including blood substitutes that are being extensively developed by many groups around the world (example of references: Chang 2007;Liu and Xiu, 2008; Mozzarelli,2010; Zapol 2012).

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