How do I see the production of engineered blood cells available for transfusion?

Olivier Garraud\textsuperscript{a,b,c,\textdagger}

\textsuperscript{a} Faculty of Medicine, University of Lyon, 42023, Saint-Etienne, France
\textsuperscript{b} Institut National de la Transfusion Sanguine, 75015, Paris, France
\textsuperscript{c} Palliative Care Unit, The Buffle Hospital, 16700, Buffle, France

ARTICLE INFO

Keywords:
blood components
in vitro
engineering
red blood cells
platelets

ABSTRACT

The in vitro production of red blood cells and platelets is a groundbreaking technology that can—when optimized—surrogate for donated blood cells, in total or in part. Here we discuss questions that may arise when the technology is available, relative to safety issues (comprising both quantitative and qualitative parameters) and to ethics, an item often forgotten in the debates so far.

1. Introduction

Hemoglobin (Hb) is a molecule that is extremely complex in terms of biochemistry; this renders its engineered synthesis nearly impossible. Animal Hb proved unsuccessful in binding iron to be delivered to human tissues. Artificial oxygen (O\textsubscript{2}) carriers have been injected for decades with—again—little success, and numerous complications \cite{1,2}. Recent hopes rely on a novel O\textsubscript{2} carrier originating from a marine worm. The process—termed HEMOXYCarrier\textsuperscript{™} (Hemarina, Morlaix, France)—is based upon the great capacity of extracellular Hb extracted from the marine worm \textit{Arenicola marina} to satisfactorily restore tissue oxygenation without leading to adverse events. This technology revolves around the hemoglobin found in the marine worm \textit{Arenicola marina}; its hemoglobin is very similar in structure to that found in humans, but differs by its extra-cellular nature. As it is not contained within red-blood cells, it is thus compatible with all blood groups. Further, it is capable of binding 40 times more oxygen than human hemoglobin. And last, it is 250 times smaller than human red blood cells, allowing exquisite diffusion in vessels \cite{3}. This Hb is assumed to be neither allergenic nor immunogenic, according to the manufacturer.

For the past fifteen years, human red blood cells have been produced in vitro \cite{4}; programs to produce human erythrocytes use diverse sources, in particular: pluripotent stem cells (PSCs); embryonic stem cells (ESCs); induced pluripotent stem cells (iPSCs); umbilical cord blood (UCB); peripheral blood (PB); and hematopoietic stem/progenitor cells (HSPCs), as reviewed in \cite{5}. In vitro generated red blood cells are now evaluated in clinical trials. There is the hope, at least raised by investigators, that production can surrogate donated cells to transfuse patients in need \cite{6}; this raises a number of technical \cite{7}, and also ethical, issues that I discuss later on.

The availability of platelet components is even more difficult than of erythrocytes, because of the short preservation time of around 5 days (3–7 days, depending on the process and the level of safety wished at 22 ± 2 °C) \cite{8,9}; 4 °C platelets are now made available for resuscitation, and active bleeding emergency, protocols \cite{10}, but this temperature does not suit preventive transfusion in persons at risk of bleeding because of severe thrombocytopenia and also in patients presenting with platelet dysfunctions. Several programs to generate in vitro platelets suitable for transfusion programs have been launched throughout the planet, with very little success so far, despite hopes; in vitro generated platelets derive from: HSCs, HPs, iPSCs, ESCs, and immortalized megakaryocytes (iMK), from cord blood (CB) or PB \cite{11,12}. The in vitro production of platelets remains, however, very disappointing in terms of quantitation; it is too early to evaluate quality at this stage, despite some authors’ claims \cite{13-15}.

Questions relative to the clinical use of in vitro engineered red blood cells and platelets are nevertheless largely similar, and can be challenged—in my personal view—in a SWOT analysis.

2. Strengths

The in vitro production of red blood cells and platelets is groundbreaking technology. Combined with another innovative technology, i.e. the manufacturing of “universal” stem elements, it should allow for
the production of blood cells lacking the most immunizing moieties and represent a solution to solve situations of multi-immunization and transfusion dead-ends [16,17].

While the latter would stand for universal blood, another issue is the extreme individualization of blood cell manufacturing suited to rare blood groups (absence of public antigens and/or the presence of private antigens) [18,19].

On those grounds, in vitro production of blood cells would need to be an exquisite personalized (transfusion) medicine.

3. Weaknesses

The in vitro production of red blood cells is possible and the produced cells proved safe in a preclinical trial [NCT0929266]. Recommendations have been made to move forward to an industrialized scale up 1 [20,21]. However, mass production is not-yet achievable; this would require considerable investment and efforts. Regarding platelets, this status is far from being satisfactorily achieved.

When available for clinical use, and contrary—in my opinion—to what is frequently claimed (in position papers), the transfusion-transmitted infectious risk is not completely overcome; indeed, HSCs, iPSCs or even ESCs may contain endogenous (retro)viruses that can, in theory, be amplified with no regard to long-term outcome, especially as the endogenous retroviruses are regarded as potential innate immune makers and are capable of self-protecting against foreign infections [20,21] but their effect on a foreign body has never been considered to the best of my knowledge and might, perhaps, be important, in spite of the fact that no related pathology has been reported following allo- genous stem cell transplantation. However, abnormal activation of human endogenous retroviruses (HERVs) has been associated with several diseases such as cancer, autoimmunity, and neurological disorders [22]. Of important note, when pathogen reduction/inactivation technologies are validated for clinical use for red blood cell concentrates, this concern may be reduced unless some viruses resist the process.

Further, storage lesions—which appear to be responsible for a non-negligible percentage of adverse transfusion reactions in recipients [23–27]—would not be prevented; plastics, pipes, unnatural gas exchange, anticoagulants, buffers, temperatures, all differ from physiological conditions stricto sensu and may each (or in combination) create effects on the recipients’ vascular endothelial cells, on the recipients’ own circulating cells, and perhaps on tissues such as in the lung in case of extravasation. This will have to be scrutinized further when pathogen reduction/inactivation technologies are applied to red blood cells, as this process may add its own storage lesions [28].

Next, an issue which is also barely addressed is the age of red blood cells (this will be also the case for platelets when available); indeed, a transfused blood component comprises virtually equal fractions of red cells of each age from 1 day to 120 days, as present in the donor’s circulating volume. Each day, the expiring fraction, estimated to be 1/120, is naturally eliminated; actually, a much larger fraction is destroyed daily because transfused red cells do not survive as long as their native counterpart [29,30]. By all means, however, it is expected that the transfused component survives in a Gaussian pattern and does not collapse abruptly because it is synchronized at the beginning (to avoid an abrupt lack of oxygenation and also the release of toxins, such as free Hb and iron). This would mean that, optimally, in vitro generated blood components would be composed of a mixture of fractions of different age; this is expected to complexify the production and quality control processes.

Last, adverse reactions specific to those components are yet unknown; a balance between a reduction of certain adverse reactions, e.g. linked to immunological incompatibility, and the appearance of novel ones is to be anticipated. As there is no specific new item to monitor, this will then complexify hemovigilance and the surveillance of transfusions [31].

4. Opportunities

When technically feasible the in vitro production of red blood cells and platelets on a relatively large scale would be an option to maintain a suitable inventory of blood components to face crises—such as an epidemic outbreak as seen on different occasions (WNV, Chikungunya, Zika, Dengue…) or a pandemic event such as recently seen with the SARS-Cov-2 infection (COVID-19)—. This would also allow the maintenance of e.g. a safety inventory of group O, RhD negative (Rh-1), RhC and RhE negative (RH-2,-3,4,5) red blood cells. Contrary to the situation exposed in a preceding section and referred to as a “Strength”, that was relative to qualitative properties, this one would refer to quantitative safety.

5. Threats

Disruptive technologies are mainly developed by and for industrialized countries, especially when they represent an immense financial effort. Despite that, in theory, industries can prepare transfusion grade blood components with in vitro generated cells and ship them to clients i.e. blood transfusion services in remote countries. It is obvious that the number of barriers is also immense to afford this globalized activity at an affordable cost and within the accepted quality range. Access to such engineered activity would likely increase the gap between Northern and Southern countries, and oppose the ethical principal of justice.

In Northern countries, this will further question the now accepted model of Voluntary Non- Remunerated Blood Donation [32]. Will this donation mode coexist with the industrial process? Will conventional blood donation persist and under which governance? Would the development of engineered blood components ease, or, on the contrary, brake the development of the VNRD model in the South as wished for by the WHO and the majority of NGOs, official bodies and blood transfusion systems nowadays?

Further, what will be the economic model for accessing source cells, i.e. progenitor cells (of any type)? Will “original” blood cells be patented, with benefits to the industry and likely not for the genetic owner of the cell? In other words, who will own the in vitro generated cells? Indeed, given that blood for transfusion purpose is now largely, though with some debate, considered a public resource [33–36], plasma is often considered a private one, that can be obtained from individuals for a financial reward [37,38].

The disruptive technology of in vitro generated blood cells will also certainly represent a paradigm change in transfusion medicine, through the ownership or the public characteristic of blood.

6. Concluding remarks

Each of the alternatives thought of to replace human blood in transfusion programs has merits and caveats, to solve either quantitative or qualitative (phenotype) problems. Once problems are identified, solutions might be found to render those processes applicable in the routine. It is my personal opinion that solutions may be universal as universality is the motto of each of the disruptive technologies considered; they must be universal to serve all interests, in economically wealthy systems as well as in intermediate or underdeveloped economies, as it would be unacceptable to leave e.g. African countries struggling with making a blood component inventory when the ethical model of volunteer donation is destroyed. I am eager to see what solutions are found by promoters of tomorrows’ transfusions, to make it safe to beneficiaries and affordable to healthcare providers and tax payers.

1 20-21
Disclosures

I am an occasional consultant for Cerus Europe, Amersfoort, NL.

References

[1] Chen JY, Scerbo M, Kramer G. A review of blood substitutes: examining the history, clinical trial results, and ethics of hemoglobin-based oxygen carriers. Clinics (Sao Paulo) 2009;64:803–13.

[2] Mozzarelli A, Rondi L, Faggiano S, Bettati S, Bruno S. Haemoglobin-based oxygen carriers: research and reality towards an alternative to blood transfusions. Blood Transfus 2010;8:59–68.

[3] Le Gall T, Polard Y, Routelot M, Lotte A, Rauziene M, Lehn P, et al. In vivo biodistribution and oxygenation potential of a new generation of oxygen carrier. J Biotechnol 2014;187:1–9.

[4] Giarratana MC, Kobari L, Lapillonne H, Chalmers D, Kiger L, Cyanober T, et al. Ex vivo generation of fully mature human red blood cells from hematopoietic stem cells. Nat Biotechnol 2005;23:69–74.

[5] Solves Alcaina P. Platelet Transfusion: And Update on Challenges and Outcomes. J Blood Med 2020;11:19–32.

[6] Douay L. Why industrial production of red blood cells from stem cells is essential for tomorrow’s blood transfusion. Regen Med 2018;13:627–32.

[7] Rousseau GF, Giarratana MC, Douay L. Large-scale production of red blood cells from stem cells: what are the technical challenges ahead? Biotechnol J 2014;9:28–38.

[8] Humbrecht C, Kientz D, Gachet C. Platelet transfusion: current challenges. Transfus Clin Biol 2018;25:151–64.

[9] Humbrecht C, Kientz D, Gachet C. Platelet transfusion: current challenges. Transfus Clin Biol 2018;25:151–64.

[10] Cap AP, Reddoch-Cardenas KM. Can’t get platelets to your bleeding patients? Just chill... the solution is in your refrigerator! Transfus Clin Biol 2018;25:217–9.

[11] Di Buduo CA, Kaplan DL, Baldusini A. In vitro generation of platelets: Where do we stand? Transfus Clin Biol 2017;24:273–6.

[12] Strassel C, Gachet C, Lanza F. On the way to in vitro platelet production. Front Med 2018;5:239.

[13] Moreau T, Evans AL, Vasquez L, Tijssen MR, Yan Y, Trotter MW, et al. Large-scale production of megakaryocytes from human pluripotent stem cells by chemically defined forward programming. Nat Commun 2016;7:11208.

[14] Vainchenker W, Raslova H. Megakaryocyte polyploidization: role in platelet production. Platelets 2019;9:1–10.

[15] Zimring JC, Hudson KE. Cellular immune responses in red blood cell alloimmunization. Hematol Am Soc Hematol Educ Program 2016;2016:452–6.

[16] Garraud O, Cognasse F, Moncharmont P. Immunological features in the process of blood platelet-induced alloimmunization, with a focus on platelet component transfusion. Diseases 2019;7:7.

[17] Khan J, Delaney M. Transfusion support of minority patients: extended antigen donor typing and recruitment of minority blood donors. Transfus Med Hemother 2018;45:271–6.

[18] Frazier S, Higgins J, Bugajski A, Jones A, Brown M. Adverse reactions to transfusion of blood products and best practices for prevention. Critical Care Nurs Clin North Am 2017;29. https://doi.org/10.1016/j.cccn.2017.04.002.

[19] Haddad A, Bou Assi T, Baz E, Samaha H, Hachem B, Feghali R, et al. Transfusion-associated hazards: a revisit of their presentation. Transfus Clin Biol 2018;25:118–35.

[20] Rebullia P. The long and winding road to pathogen reduction of platelets, red blood cells and whole blood. Br J Haematol 2019;186:655–67.

[21] Prost M, Tinot JD, Lion N. In vitro assays and clinical trials in red blood cell aging: lost in translation. Transfus Apher Sci 2015;52:279–97.

[22] Zolla L, D’angelo A, Rinalducci S, D’Amici GM, Papella S, Baglio S, et al. Classic and alternative red blood cell storage strategies: seven years of “omics” investigations. Blood Transfus 2015;13:21–31.

[23] Garraud O. How to reposition the benefit-risk balance to safely transfuse patients in non-vital situations? Transfus Clin Biol 2019;26:171–3.

[24] Haddad A, Bou Assi T, Baz E, Samaha H, Hachem B, Feghali R, et al. Blood donations mode: assessment of the Lebanese model. Transfus Clin Biol 2019;26:341–334.

[25] Flannagan P. The code of ethics of the international society of blood transfusion. Blood Transfus 2015;13:537–48.

[26] Farrugia A, Del Bò C. Some reflections on the code of ethics of the international society of blood transfusion. Blood Transfus 2015;13:551–8.

[27] Farrugia A, Del Bò C. Reply to Flanagan “The Code of Ethics of the International Society of Blood Transfusion” [Blood Transfus 2015;13:537–48]. Blood Transfus 2017(15):286–8.

[28] Garraud O. Let us rejoice in the remarkable persistence of transfusion but remain alert to the risks in ambush. Transfus Clin Biol 2020;27(2).

[29] Mercier Y. The contested market of plasma. Transfus Clin Biol 2020;27:52–7.