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

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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_2) carriers have been injected for decades with— again—little success, and numerous complications [1,2]. Recent hopes rely on a novel O_2 carrier originating from a marine worm. The process—termed HEMOXYCarrier™ (Hemarina, Morlaix, France)—is based upon the great capacity of extracellular Hb extracted from the marine worms *Arenicola marina* to satisfactorily restore tissue oxygenation without leading to adverse events. This technology revolves around the hemoglobin found in the marine worm *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 [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 [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 [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 [6]; this raises a number of technical [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) [8,9]; 4 °C platelets are now made available for resuscitation, and active bleeding emergency, protocols [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, iPS, ESCs, and immortalized megakaryocytes (iMK), from cord blood (CB) or PB [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 [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 ground breaking technology. Combined with another innovative technology, i.e. the manufacturing of "universal" stem elements, it should allow for

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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, iPS 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 allogenous 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 reducedunless 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 up 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 promotors 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, Amersfoot, NL.

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