Effect of Induced Pluripotent Stem Cell Technology in Blood Banking

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SUMMARY

Population aging has imposed cost-effective alternatives to blood donations. Artificial blood is still at the preliminary stages of development, and the need for viable cells seems unsurmountable. Because large numbers of viable cells must be promptly available for clinical use, stem cell technologies, expansion, and banking represent ideal tools to ensure a regular supply. Provided key donors can be identified, induced pluripotent stem cell (iPSC) technology could pave the way to a new era in transfusion medicine, just as it is already doing in many other fields of medicine. The present review summarizes the current state of research on iPSC technology in the field of blood banking, highlighting hurdles, and promises. STEM CELLS TRANSLATIONAL MEDICINE *2016;5:269–274*

SIGNIFICANCE

The aging population in Western countries is causing a progressive reduction of blood donors and a constant increase of blood recipients. Because blood is the main therapeutic option to treat acute hemorrhage, cost-effective alternatives to blood donations are being actively investigated. The enormous replication capability of induced pluripotent stem cells and their promising results in many other fields of medicine could be an apt solution to produce the large numbers of viable cells required in transfusion and usher in a new era in transfusion medicine. The present report describes the potentiality, technological hurdles, and promises of induced pluripotent stem cells to generate red blood cells by redifferentiation.

CURRENT AND FUTURE NEED FOR BLOOD COMPONENTS

Red blood cell (RBC) transfusion is the main therapeutic option for acute hemorrhages. This assumption has led the World Health Organization to include blood within the Model List of Essential Medicines, point 11.1 [1]. In accordance with the World Health Assembly resolution, WHA63.12, the World Health Organization has recognized that achieving self-sufficiency, unless special circumstances preclude it, in the supply of safe blood components based on voluntary, nonremunerated blood donation, and the security of that supply are important national goals to prevent blood shortages and meet the transfusion requirements of the patient population.

Progressive aging of the population in Westernized countries has two immediate dismal consequences: fewer blood donors and more blood recipients. The Finnish transfusion registry data have demonstrated a marked increase in RBC consumption with increasing age among recipients, beginning at approximately 50 years of age. Those aged 70 to 80 years have an eightfold higher RBC consumption than those aged 20 to 40 years [2].

FROM BLOOD CELL SUBSTITUTES TO STEM CELL-DERIVED BLOOD CELLS

Artificial oxygen carriers or recombinant hemoglobin-based oxygen carriers tested until now cause vasoconstriction triggered by nitric oxide scavenging and/or oxygen oversupply in the precapillary arterioles. To date, for clinical purposes, one must still rely on whole cells from accurately screened donors. In order to achieve meaningful clinical benefit, a transfusion unit must contain approximatively 10^{12} RBCs, or $300–600 \times 10^9$ platelets. Such a huge number of cells makes transdifferentiation from human adult somatic cell types a scientific exercise with good in vitro results [3] but poor clinical potential.

Currently, advances in genetic engineering have made it possible to knock out the genes of multiple xenoantigens, such as galactose α-1,3-galactose and N-glycolylneuraminic acid. Nevertheless, usage of xenogeneic RBCs from genetically engineered pigs still has major hurdles, leading to rapid clearance from circulation when transfused into nonhuman primates [4]. Thus, only two technologies have been deeply investigated to date for producing universal RBCs.

Modification of Blood Group Antigens Includes Two Basic Methods

First, group O RBCs are safe for transfusion to persons of any ABO blood group, because group O cells bear H chains without terminal A or B monosaccharides. In theory, group O RBCs could be created from cells of another ABO blood group, because the A and B terminal immunodominant sugars (120,000–1,170,000 sites per cell for group A and 610,000–830,000 for group B) could be removed using specific exoglycosidases. This treatment produces

enzyme-converted group O RBCs [5]. A successful phase II trial using recombinant enzyme was reported in 2000 [6] but development later stopped. Because of the complexity of group A antigens, conversion of group A RBCs, in particular, A_1 RBCs, to group O RBCs is more difficult. However, a new bacterial glycosidase efficiently cleaving antigens on the surface of both A_1 and A_2 RBCs has been obtained [7].

Second, pegylation camouflages the antigens on the surface of RBCs with nonimmunogenic molecules such as polyethylene glycol (PEG) in a nonspecific manner, to provide O, minor antigen-negative phenotype RBCs. Recent findings that PEG is immunogenic in animals and humans and that PEG antibodies can shorten the survival of PEG-RBCs are disturbing, suggesting that "stealth" RBCs might never become a reality [8]. Neither alternatives formally reduce the need for donors, which is the cornerstone of future problems to come.

Generating Universal RBCs From Progenitor and Stem Cells

Proliferating erythroblasts can be expanded from umbilical cord blood (CB) mononuclear cells ex vivo for 10^6–10^7-fold (in ∼50 days) before proliferation arrest. However, the Hayflick number is too low for achieving clinical useful doses, and, unfortunately, the efficiency of terminal differentiation is low [9]. Similarly, human erythroid progenitor cell lines immortalized by transduction of c-MYC and BCL-X_L [10] are able to produce enucleated RBCs at low efficiency, and immortalization for autologous use is difficult to achieve [11].

Stem cells are the factor that is really needed [12, 13]. Robust bone marrow (BM) is able to produce a daily count of 2.5 billion RBCs, 7 million platelets, and 850,000 granulocytes per kilogram. Thus, hematopoietic stem cells (HSCs) from peripheral blood [14, 15], BM, or CB [16] were investigated first. The usual protocol was based on inducing massive proliferation of HSCs (up to 1.95×10^6-fold), followed by high-efficiency (95%) terminal erythroid differentiation. However, harvesting HSCs has obvious time and cost limitations when planning autologous use.

Pluripotent stem cells, able to differentiate into any of the three germ layers, can be isolated from blastocysts (embryonic stem cells [ESCs]) or generated by reprogramming of adult somatic cells (induced pluripotent stem cells [iPSCs]) [17] (Fig. 1). In a leading industrial trial, Ocata Therapeutics, Inc. (Worcester, MA, http://www.ocata.com) first reported that ESCs differentiated into functional oxygen-carrying erythrocytes on a large scale (10^{10}–10^{11} cells per six-well plate of human ESCs) as a source for clinical grade mass production of RBCs [18]. Similarly, several groups have reported that ESC-derived platelets are functional in vitro and in the microcirculation of living mice [19–21].

Although ESCs represent the most promising type of cells for scientific and clinical applications [22, 23], their use poses a set of ethical concerns. In contrast, iPSCs have great potential to provide an inexhaustible source of progenitors for the generation of large numbers of RBCs and to facilitate the innovative development of allogeneic and rare blood group products for transfusion purposes.

iPSCs were generated for the first time from murine fibroblasts in 2006 by Takahashi and Yamanaka using ectopic expression of transcription factors Oct4, Klf4, Sox2, and c-Myc (OKSM) [24]. In 2007, Takahashi et al. and Yu et al. successfully reprogrammed primary human fibroblasts using the OKSM cocktail [25] and Klf4, Oct4, Sox2, and LIN28 [26], respectively. Because of transformation concerns, many groups have replaced the use of the proto-oncogene c-Myc [27, 28] with less dangerous genes [29, 30] to increase the safety of delivery approaches and provide tightly reprogramming factors controllable expression systems [31, 32]. Tumorigenicity of undifferentiated iPSCs contaminating the final product is a concern that could be addressed using product irradiation or other clinically approved technologies that kill pathogens and nucleic acid-containing cells [33, 34].

iPSC-derived, pathogen-free, autologous or universal blood cells have the potential to alleviate allogeneic supply shortages. Small-scale bioreactors with disposable kits (e.g., Quantum Cell Expansion System; Terumo BCT, Lakewood, CO, http://www.terumobct.com; or Xuri; General Electric, Stanford, CT, http://www.ge.com) allow for in-hospital expansion of suspension cell cultures [35]. On an industrial size, large-scale bioreactors allow bulk production of iPSCs in the desired numbers and potentially with no Hayflick limit.

Most methods rely on mouse embryonic fibroblast (MEF) feeders and serum at some point during their culture. Because both MEF and serum can potentially be contaminated with xenogeneic pathogens, their use increases the risk to recipients; hence, serum-free and xeno-free protocols are being developed for generation of iPSCs [36] and redifferentiation to blood cells [37]. Redifferentiation of iPSCs to mature blood cell types seems the most difficult step in blood cell manufacturing from iPSCs [38]. In vitro redifferentiation is based on sequential addition of cytokines at defined concentrations [38] (summarized in Fig. 2).

Redifferentiating iPSCs Into RBCs

The major limitations for translating iPSC-derived RBCs into the clinic are (a) inefficient enucleation, (b) difficulty switching to the adult-type (β) globin form, and (c) the possibly insurmountable number of RBCs (10^{12}) needed to generate 1 unit. Transfusing iPSC-derived RBCs is obviously safer (and faster) than transplanting genetically engineered iPSC-derived HSCs but has two major limitations: a short half-life and the need for repeated, lifelong transfusions.

A number of groups [39–41] have reported successful differentiation of iPSCs down the erythroid lineage using a variety of culture systems (stromal feeder-dependent or -independent), generating orthochromatic erythroblasts and reticulocytes (up to 10%), although Kobari et al. [42] have reported up to 26% enucleation for erythroid cells differentiated from iPSCs from an individual with sickle cell disease. The differentiated cells in all reports expressed fetal and embryonic globins, indicating reprogramming of the globin locus from the original parental cell. Erythroid differentiation has been confirmed by morphological analysis and the expression of a very limited number of RBC markers, including glycophorin A and transferrin receptor [40, 41]. Functionally, Kobari et al. [42] have shown that the reticulocytes generated from iPSCs exhibit an oxygen-binding capacity similar to that of CB RBCs, which contain predominantly fetal hemoglobin. Trakarnsanga et al. showed that the proteome of erythroid cells differentiated
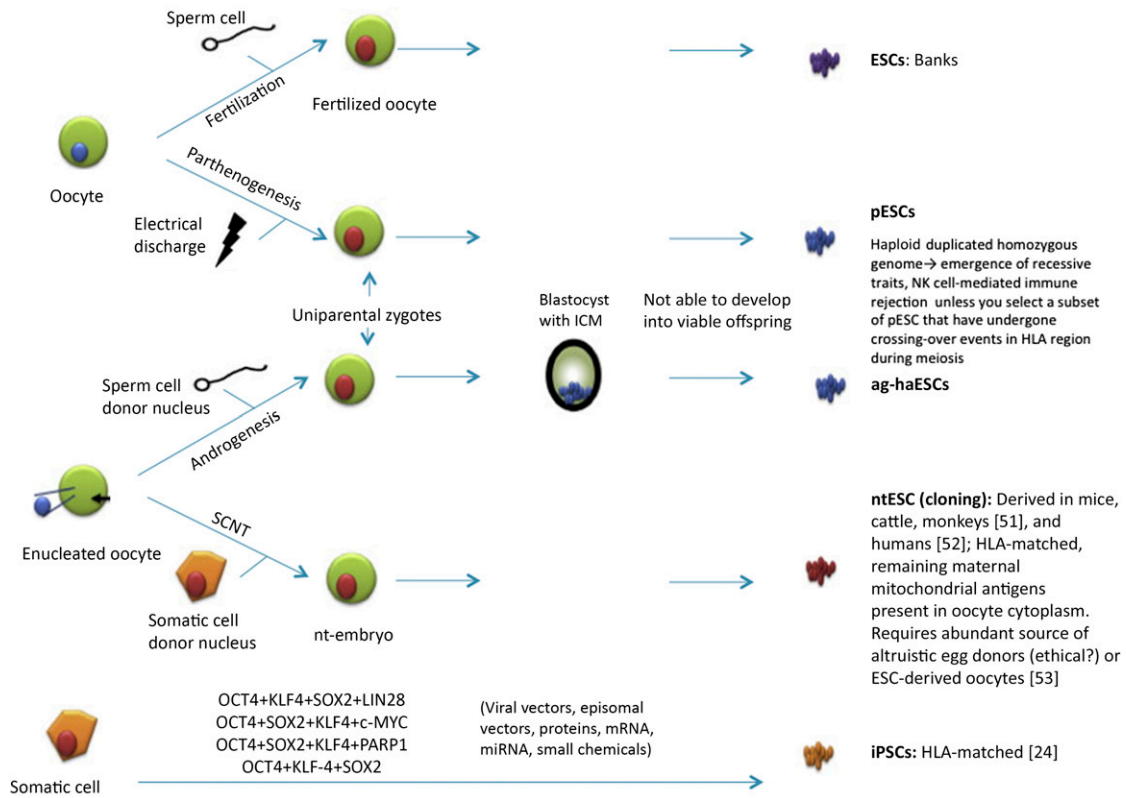
Figure 1. Different strategies to generate pluripotent stem cells [24, 51–53]. Abbreviations: ag-haESC, androgenetic haploid embryonic stem cell; ESC, embryonic stem cell; HLA, human leukocyte antigen; ICM, inner cell mass; iPSC, induced pluripotent stem cell; miRNA, micro-RNA; NK, natural killer; nt, nuclear transfer; pESC, parthenogenetic embryonic stem cell; SCNT, somatic cell nuclear transfer.

from iPSC lines is 98% similar to that of normal adult erythroid cells [43].

The iPSC erythroid cells expressed γ- but little β-globin, likely owing, at least in part, to the low level of KLF1 and the absence of BCL11A in these cells, both of which are known to be required for the developmental switch from fetal to adult globin expression [44, 45]. Apart from proteomics and immunophenotype data, studies addressing the function of iPSC-derived RBCs (e.g., the capacity to release oxygen to tissue under normoxia and hypoxia) in vivo are lacking; this should be the focus of investigation in the future.

Redifferentiating iPSCs to Platelets

The production of pathogen-free O donor platelet concentrates with negligible isoagglutinin titers would be the ideal aim. iPSC-derived platelets have been generated [35, 46] and Advanced Cell Technology, Inc. (Worcester, MA) is investing in clinical trials. Feng et al. recently reported a serum and animal feeder-free method that permits differentiation of human iPSCs into megakaryocytes (MKs) and functional platelets in less than 20 days and cryopreservation of MK progenitors, enabling a rapid "surge" capacity when large numbers of platelets are needed. iPSC-derived platelets form aggregates, lamellipodia, and filopodia after activation and circulate in macrophage-depleted animals and incorporate into developing mouse thrombi in a manner identical to human blood platelets. By knocking out the β_2-microglobulin gene, platelets that are

negative for class I human leukocyte antigen (HLA) have also been generated [37, 47].

BLOOD BANKING AND TRANSFUSION

The limited shelf life of primary RBCs and platelets is likely to also remain a problem with iPSC-derived blood cells; however, having a precursor available will allow periodic reconstitution of the master cell bank. Because of the short half-life and absence of a nucleus, investigators and recipients will be spared from concerns regarding reprogramming-induced oncogenicity. Efficient screening to accurately separate all the anucleated cells from the nucleated counterpart appears to be a limiting step for this type of application.

The currently available methods for the production of iPSCs are extremely inefficient and costly, and achieving off-the-shelf universal RBCs from a few key universal donors (homozygous at most relevant loci) would be the preferable approach [48]. The characteristics of the top three most useful universal donors are listed in Table 1 [48].

Although group O RBCs are considered universal, they are still immunogenic for recipients with the Bombay phenotype. The Bombay phenotype is one of the rare phenotypes in the ABO blood group system that fails to express ABH antigens on RBCs and develops natural anti-O isoagglutinins. Nonsense or missense mutations in fucosyltransferase 1 (*FUT1*) and 2 (*FUT2*) genes are known to create this phenotype. This blood
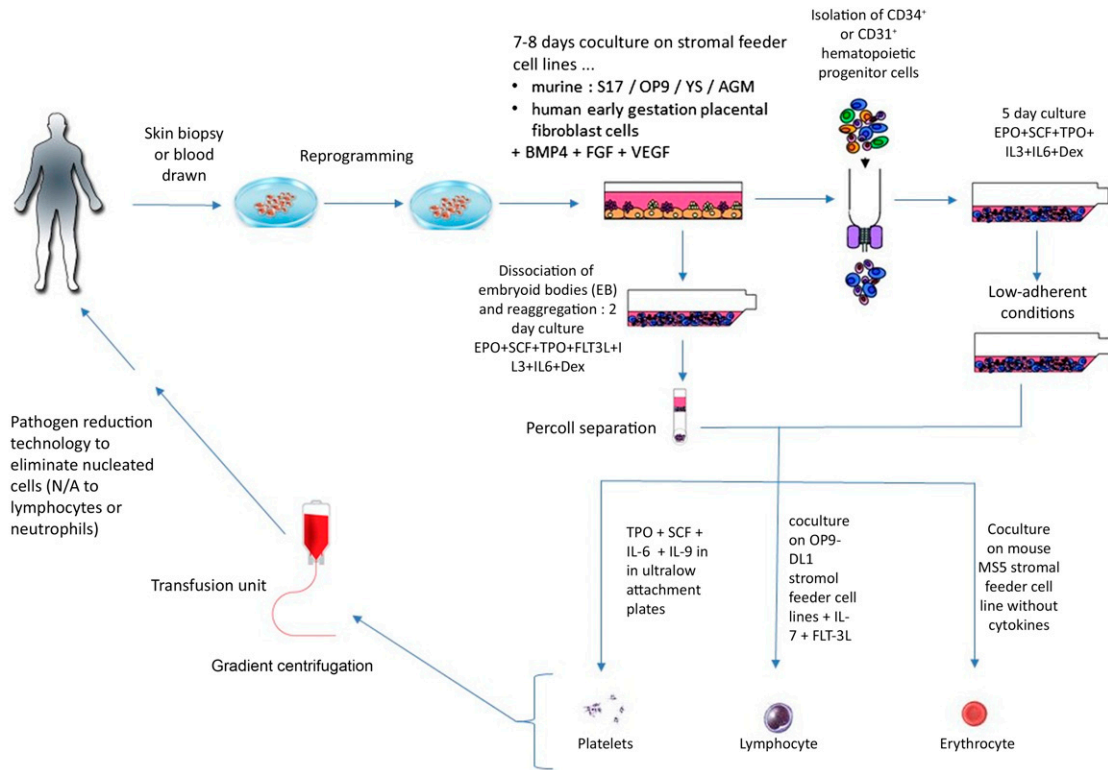
Figure 2. Different strategies to redifferentiate iPSCs to RBCs or platelets [35, 39-42, 46]. Abbreviations: BMP4, bone morphogenetic protein 4; Dex, dexamethasone; EPO, erythropoietin; FGF, fibroblast growth factor; FLT3L, FLT3 ligand; iPSCs, induced pluripotent stem cells; IL, interleukin; N/A, not applicable; SCF, stem cell factor; TPO, thrombopoietin; VEGF, vascular endothelial growth factor.

Table 1. Phenotype of induced pluripotent stem cell clones that would be theoretically useful for the management of alloimmunized recipients

		Systems																
		RH					KEL		Fy		Jk		MNS				LU	KEL	P1	
Variable	ABO type																			Cumulative coverage (%)
Antigens		D	C	E	c	e	K	k	a	b	a	b	M	N	S	S	1	Kpa	1	
Clone 1	O	−	−	−	+	+	−	+	−	+	−	+	−	+	−	+	−	−	−	95.53
Clone 2	O	+	+	−	−	+	−	+	−	+	+	−	+	−	−	+	−	−	−	+3.14 (= 98.67)
Clone 3	O	−	−	−	+	+	−	+	+	−	+	−	−	+	−	+	−	−	−	+0.46 (= 99.13)

Reported coverage applies to French population of recipients (adapted from Table 1 of Peyrard et al. [48]).

group is compatible with all other blood groups as a donor, because it does not express the H antigen on RBCs. Jackman et al. reported in 2010 the establishment of human iPSCs from the dermal fibroblasts of a Bombay blood type individual [49].

However, universal ABO compatibility is not the only requirement, because many other different antigenic systems expressed on RBCs are immunogenic. Once alloimmunization occurs, such patients require RBCs from donors with a different blood group antigen combination, making it a challenge to find donors after every successive episode of alloimmunization. Peyrard et al. retrospectively studied a cohort of 16,486 consecutive alloimmunized patients (10-year period), showing 1 to 7 alloantibodies with 361 different antibody combinations [48]. They showed that only three human iPSC clones (those reported in Table 1) would be sufficient to match more than

99% of the 16,486 patients in need of RBC transfusions. The study of the French National Registry of People with a Rare Blood Phenotype/Genotype (10-year period) showed that 15 human iPSC clones would cover 100% of the needs in white patients. In addition, one single human iPSC clone would meet 73% of the needs of alloimmunized patients with sickle cell disease for whom rare cryopreserved RBC units are required. Hence, a very limited number of RBC clones would be able to not only provide for the need for most alloimmunized patients and those with a rare blood group but also to efficiently allow for a policy for alloimmunization prevention in multiply transfused patients [48].

ABO compatibility is also definitively not the only requirement for platelet transfusions. Even with platelet transfusion, other antigen systems (i.e., HLA and human platelet glycoprotein antigen) keep the risk of alloimmunization high.

Table 2. Advantages from mass production of universal RBCs from a single donor vs. current approach to blood collection and banking

Parameter	Current approach	Future approach (universal blood cells from selected donors achieved via iPSC expansion)
Cost of medical selection of donors	Dedicated personnel for each donation, every day	Only once at startup
Cost of blood processing	Collection (including nurses), fractionation (including laboratory technicians) at many peripheral collection points/hospitals	Dedifferentiation, expansion in bioreactors and redifferentiation at a single industrial facility
Cost for transportations	From every collection center to the processing center daily and from processing center to transfusion centers every day	From processing center to transfusion centers every several days
Cost of NAT and serological screening	On each donation	Only once on master cell banks
Cost of other release tests	Complete blood counts and clinical chemistry at each donation	Tumorigenicity as batch release test
Cost of leukodepletion	Each blood component	Not needed
Cost of pathogen inactivation techniques or irradiation	Each blood component	Although theoretically needed only once on the source material, it might be preferred at the end of redifferentiation to kill viable (undifferentiated) iPSCs with tumorigenic potential
Risk of major transfusion error	Low but persisting for non-O units	Absent (only O units)
Risk of erythrocyte antigen sensitization	High; requires extensive donor and recipient antigen typing and matching	Absent (few clones for almost universal coverage)
Risk of HLA sensitization from granulocyte concentrates	Requires HLA-matched donors or donor HLA typing and screening for donor-specific anti-HLA antibodies	Absent
Risk of HLA sensitization from platelet concentrates or contaminating white blood cells	Reduced but not totally prevented by leukoreduction; requires HLA-matched donors or donor HLA typing and screening for donor-specific anti-HLA antibodies	Absent for HLA-less platelets
Cost of quality assurance (site, personnel and instrument qualification; process validation)	Each site	Single site

Abbreviations: HLA, human leukocyte antigen; NAT, nucleic acid test; RBC, red blood cell.

However, in this setting, published studies reporting combined antigen frequencies, which is the prerequisite to identify the coverage achievable with key "universal" donors, are also lacking.

CONCLUSION

Blood cells are the ideal candidates for clinical trials of iPSC-redifferentiated cell therapies. The proteome of blood cells differentiated from iPSC lines is very similar to that of normal adult blood cells; however, further work to improve the induction of erythroid cells in existing iPSC lines is required before iPSC-derived RBCs are suitable for transfusion therapy. Engineering technology such as bioreactor use might be necessary for successful advances; however, the details and cost will remain the main difficulties. If these can be overcome, we will realize several advantages (summarized in Table 2) in terms of ease of availability and costs to the health care system [50].

AUTHOR CONTRIBUTIONS

D.F.: conception and design, manuscript writing; M.P.: manuscript writing.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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