Reshaping military medicine: the pivotal role of bioengineering in enhancing transfusible blood products

Redéfinir la médecine militaire : Le rôle clé de la bioingénierie dans l'amélioration des produits sanguins transfusables

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Abstract

In the realm of military medicine, resuscitation with blood products in patients with traumatic hemorrhage is lifesaving. Traditional blood collection processes, although effective, are hampered by issues including donor availability, risk of disease transmission, and logistical hurdles in remote destinations. This article examines technological advancements in bioengineered blood products that promise to reshape trauma care. It highlights innovative developments in cellular reprogramming and *in vitro* generation of red blood cells from stem cells that hold the promise of an inexhaustible blood supply, critically important in military conflict areas. The generation of cultured platelets through advanced bioengineering techniques is also explored, addressing the limitations of donor-derived platelets with short shelf-life. The article discusses the evolving need for dry blood products such as freeze-dried plasma for hemostatic resuscitation, emphasizing its logistical advantages in the zone of military conflict. These innovations collectively drive a transformative shift in transfusion medicine and hold the promise of delivering more efficient, safer, and logistically practical alternatives to conventional blood banking practices.

Key words: bioengineering, blood transfusion, platelets, freeze-dried plasma, red blood cells

Résumé

Dans le domaine de la médecine militaire, la réanimation des patients atteints d'hémorragies traumatiques par l'administration de produits sanguins représente une intervention salvatrice. Les procédés traditionnels de collecte de sang, bien qu'efficaces, rencontrent plusieurs obstacles, notamment la disponibilité des donneurs, le risque de transmission de pathogènes et les défis logistiques inhérents aux zones reculées. Le présent article se penche sur les avancées technologiques dans le domaine des produits sanguins issus de la bioingénierie cellulaire, qui promettent de révolutionner la prise en charge des patients traumatisés. Il met en exergue des progrès innovants dans le domaine de la reprogrammation cellulaire et de la production *in vitro* de globules rouges à partir de cellules souches, ouvrant la voie à une source de sang potentiellement inépuisable, d'une importance capitale dans les zones de conflits militaires. L'article explore également la génération de plaquettes par des méthodes de bioingénierie avancées, palliant ainsi les limitations associées aux plaquettes issues de donneurs, notamment leur courte durée de conservation. Il discute de l'émergence des besoins en produits sanguins secs, tels que le plasma lyophilisé, pour la réanimation hémostatique, soulignant leurs avantages logistiques significatifs en contexte de conflit militaire. Ces innovations représentent une percée dans le domaine de la médecine transfusionnelle, promettant des alternatives novatrices plus efficaces, sécurisées et fonctionnelles aux pratiques conventionnelles de gestion des banques de sang.

Mots-clés :

Introduction

In the demanding realm of military medicine, managing hemorrhage in trauma patients depends on promptly available blood products. The maintenance of adequate supplies of blood products relies on volunteer blood donors making this process notoriously challenging and susceptible to disruption leading to inevitable blood shortage. Recent advances in biotechnology have ushered in a new era of bioengineered blood products, presenting innovative solutions to these challenges.[1-3] This article explores the latest developments in this field, focusing on the *in vitro* generation of red blood cells (RBCs) from stem cells, the creation of advanced bioengineering technologies for cultured platelets, and large-scale production of freeze-dried products for hemostatic resuscitation suitable for the military settings.

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In vitro Generation of Red Blood Cells from Stem Cells

Humans produce 200 billion RBCs every day.[4] RBCs originate from a small population of hematopoietic stem cells (HSC), which in turn, generate progenitor cells that undergo terminal differentiation, resulting in mature circulating blood cells through erythropoiesis.[4] Over recent years, significant milestones in the field of bioengineering resulted in breakthrough studies, which successfully demonstrated the conversion of stem cells into mature RBC outside of a living organism or *in vitro*. Numerous cells could be used as the source to generate erythroid cells *in vitro*: $CD34^+$ hematopoietic stem/progenitor cells (HSPCs) derived from umbilical cord blood, adult bone marrow or peripheral blood, embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs) and erythroblast progenitor cell lines.[5-8]

Although earlier studies on cultured RBCs focused on the *ex vivo* differentiation of $CD34^+$ HSPCs into mature RBCs [6,9,10] (Figure 1), recent developments highlighted certain disadvantages of this technology compared to other emerging bioengineering techniques. For instance, the variability in HSPCs proliferation potential and dependency on donor availability can pose limitations in their clinical application. On the other hand, iPSCs have unlimited expansion capabilities – major advantage for large-scale production. In addition, iPSCs can be genetically engineered to produce RBCs with desired characteristics, such as universal donor blood types, which makes this source particularly attractive for clinical applications.[10,11] iPSCs offer scalability and customization but come with increased complexity and safety concerns. For example, the pluripotent nature of iPSC and the risk of transfusion of nucleated erythrocyte precursors is associated with tumorigenesis. Further research into stem cell reprogramming is necessary to develop strategies that reduce the potential for the development of undesired side effects. Biomimicry, the practice of mimicking biological systems and processes, can be applied using three-dimensional culture systems and bioreactors. This approach will help in accurately replicating the spatiotemporal microenvironment of bone marrow niches, potentially leading to improved outcomes in *in vitro* erythropoiesis.[7] Gene editing technology may help to provide an alternative source of RBCs to patients with rare blood group types. Recent advances in clustered regularly interspaced short palindromic repeats (CRISPR)-Cas-mediated technologies have provided an efficient tool for genome editing.[12] The CRISPR-Cas9 was successfully used to bioengineer universal type O RBCs by reprogramming peripheral blood mononuclear cells. Editing the ABO gene, which determines an individual's blood type, enabled the researchers to convert blood type A to universal type O in cells lacking all the Rh antigens on the red cell membrane [12]. This advancement holds potential for enhancing the accessibility of suitable blood products for transfusion, particularly for individuals with uncommon blood types or those possessing numerous antibodies.

Irrespective of the biotechnological approaches and sources utilized for *in vitro* RBC preparation, adherence to Good Manufacturing Practices (GMP) is essential. GMP, a regulatory framework, ensures the safety, quality, potency, and effectiveness of blood products. Meticulous testing and thorough documentation of all protocols are crucial to uphold the highest standards in cell culture procedures. This practice is imperative for effective management and elimination of potential risks, such as microbial contamination and variability, thereby maintaining the safety and efficacy of cellular preparations destined for therapy.

Advanced Bioengineering Technologies and Cultured Platelets

Platelets are essential components of the blood, playing a crucial role in clotting and wound healing, particularly in combat-related trauma.[13,14] The limited shelf-life of donor-derived platelets and the risk of immunological reactions are significant hurdles in traditional transfusion methods. Advanced bioengineering technologies are being developed to create cultured platelets from multiple sources: HSPCs, human ESCs and iPSCs.[3,14,15] For example, researchers from Kyoto University, Japan, have successfully developed an immortalized megakaryocyte cell line (imMKCL) from iPSC.[16] An extremely efficient method for producing megakaryocytes involves the temporary immortalization of megakaryocyte precursors through regulated expression of transgenes, which promote cell division and halt differentiation. The use of tank bioreactors with agitated flow systems and integrated turbulence have allowed the unprecedented scale-up of imMKCL-derived platelets with functional properties comparable to donor-derived platelets in pre-clinical [17] and clinical experiments [18] (Figure 2).

Culturing platelets *in vitro* from stem cells offers numerous benefits. One key advantage is the diminished risk of tumorigenicity attributed to the lack of nuclei in platelets. Moreover, strict adherence to GMPs during *in vitro* preparation of platelets, significantly reduces the risk of infections associated with traditional methods of platelet collection, preparation, and transfusion.[19]

Implementation of gene editing techniques, such as CRISPR/Cas9, to knockdown human leukocyte antigen (HLA) class I in iPSC-derived platelets is highly advantageous. HLA class I molecules expressed on human platelets are integral to immunological recognition, facilitating the immune system's ability to differentiate self from non-self-antigens. In platelet transfusion, the presence of HLA class I molecules can lead to the development of antibodies in the recipient if these molecules are recognized as foreign. This can result in an immune reaction against the transfused platelets, reducing their efficacy and potentially leading to complications such as transfusion refractoriness. The targeted downregulation of HLA class I antigens in platelets can mitigate immunogenic responses, thereby reducing the risk of transfusion refractoriness and alloimmunization risks in recipients.[20] Therefore, platelets with the knockdown of HLA class I could serve as a universal, readily available transfusion product. Ongoing research aims to develop novel platelet-containing transfusion products with extended shelf-life, which would be particularly beneficial in the military settings where traditional platelets are not readily available due to logistical and storage challenges.

Dried Plasma and Hemostatic Resuscitation in the Military Setting

Freeze-dried plasma (FDP) has been used as an alternative to fresh-frozen plasma.[13,21] FDP is plasma that has been dried to remove water, resulting in a lightweight, stable product that can be easily transported and stored for extended periods.[21] FDP can be quickly reconstituted with water and is valuable in hemostatic resuscitation, a process of restoring blood volume and clotting capability in trauma patients. FDP is particularly useful in managing hemorrhage in battlefield situations, where rapid response is critical. Future advancements in this field may include the development of bioinspired substitutes of plasma to enhance its functionality, potentially eliminating the need for traditional plasma donation.[13]

Concluding Remarks and Future Perspectives

Recent advancements in cellular reprogramming and biotechnology described in this article are transforming transfusion medicine. These advances are also leading to the emergence of novel research domains such as nanomedicine. This new field is increasingly recognized for its potential to augment traditional transfusion practices by introducing innovative therapeutic solutions. A notable example is the development of liposome-encapsulated hemoglobin, conceptualized as an artificial oxy-

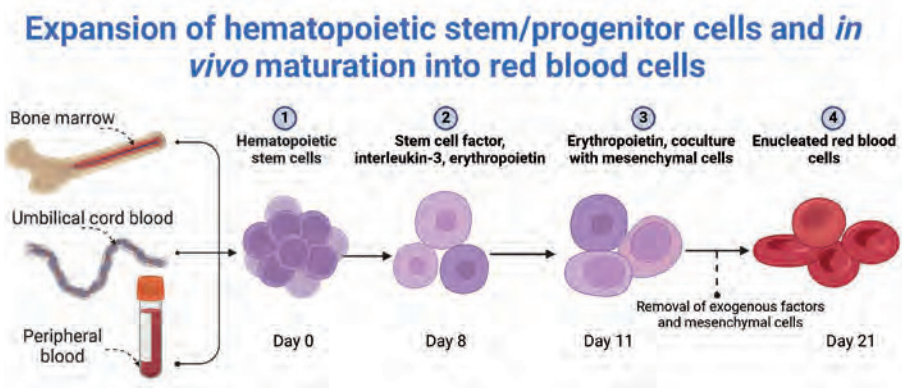

Figure 1. *In vitro* generation of red blood cells from hematopoietic stem/progenitor cells. (1) CD34⁺ hematopoietic stem/progenitor cells (HSPCs) are found in the bone marrow, umbilical cord blood and peripheral blood. Giarratana ***et al.*** isolated CD34⁺ HSPCs from peripheral blood.[6] (2) To induce a lineage-specific commitment, HSPCs were cultured for 8 days in serum-free medium in the presence of growth factors including stem cell factor, interleukin-3 and erythropoietin.[6] (3) The growth and differentiation of CD34⁺ cells was facilitated by the presence of growth factors and adherent feeder cells of murine [5] or human (bone marrow mesenchymal stromal cells) [6] origin. (4) The ***in vivo*** culture of CD34⁺ HSPCs resulted in generation of a population of erythroid cells with the morphologic, biochemical, antigenic, and functional properties of RBCs.[6]

gen carrier. This innovation aims to address oxygen deficits in the hemorrhaging patient, and strives to mimic the oxygen-carrying function of RBCs.[22] Nanomedicine also offers a groundbreaking alternative through the design of specific nanoparticles engineered to mimic the hemostatic functions of platelets. Administered intravenously, these nanoparticles facilitate efficient delivery of coagulation factors.[23]

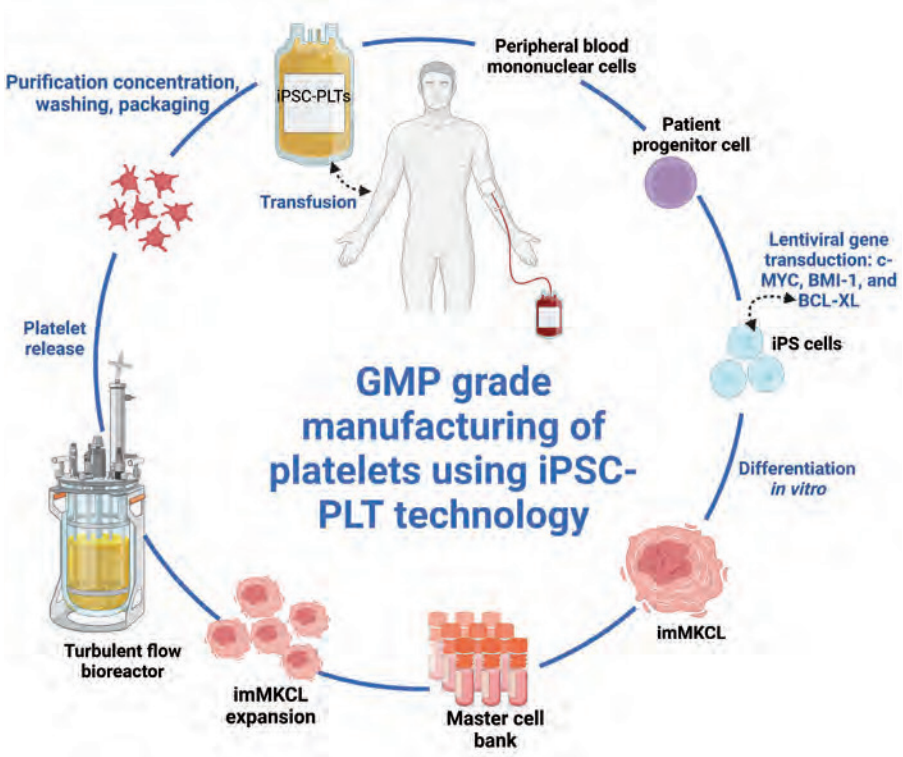

Figure 2. *In vitro* manufacture of platelets for therapeutic use from human iPSCs. Diagram showing the process established by Ito ***et al***. which enables scalable expansion and maturation of megakaryocytes from human iPSCs to generate platelets under Good Manufacturing Practice conditions.[17] For the first time, cultured platelets were successfully transfused into a patient with severe aplastic anemia.[18] Peripheral blood mononuclear cells were used to generate iPSC. Immortalized megakaryocyte progenitor cell line (imMKCL) was established using a lentival vector gene delivery and stable transduction of three Dox-inducible transgenes: c-MYC, BMI-1, and BCL-XL.[16,17] A stable imMKCL clone was selected for the generation of a master cell bank. Megakaryocytes from this master cell bank were used to produce platelets using turbulent flow bioreactors and a mixture of soluble mediators to promote megakaryocyte maturation and preserve platelet function. In this culture system, megakaryocytes release functional platelets, providing a potential source of platelets destined for transfusion.[18] ***Abbreviations:*** iPSC – induced pluripotent stem cells; imMKCL - immortalized megakaryocyte progenitor cell line, GMP - good manufacturing practice, PLT - platelets.

These emerging techniques not only promise to enhance the efficacy of conventional transfusion practices but may also provide a viable solution in circumstances where traditional blood products are unavailable. This approach is especially pertinent in the management of war-related traumatic injuries, underscoring the substantial potential of nanomedicine and other, yet to be discovered techniques, to complement the existing practices used in transfusion medicine by offering targeted, efficient, and safe treatment modalities.

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