Steady-state hematopoietic stem cells for transplantation

Hematopoietic transplantation can save the lives of many patients. However, there are still challenges about hematopoietic stem cell (HSC) collection that researchers such as Dr Zoran Ivanovic, who works for the French National Blood Service (EFS), try to overcome. Collecting HSCs by cytapheresis after mobilising them from the bone marrow is one of the most common ways to retrieve them, but Dr Ivanovic showed that the usual mobilisation treatment could be replaced by a safe ex vivo expansion of HSC collected by cytapheresis in steady-state context (i.e. without mobilisation).

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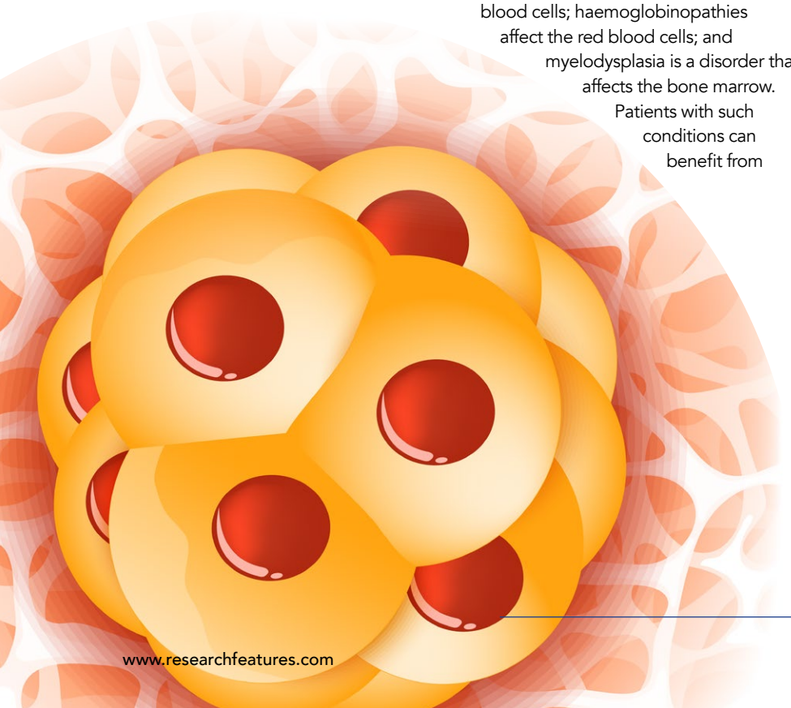


Blood contains many different cells that assume essential functions. Red blood cells, also called erythrocytes, are the most common type of blood cell. They deliver oxygen to all tissues and organs throughout the body. White blood cells, also called leucocytes, are the cells of the immune system (such as lymphocytes) that are involved in protecting the body against both infectious disease and foreign invaders. All blood cells are produced and derived from cells in the bone marrow known as hematopoietic stem cells (HSCs): HSCs have the ability to proliferate and differentiate into any type of blood cell.

Different conditions can affect blood cells. For instance, leukaemia is a cancer that begins in the bone marrow and results in high numbers of abnormal blood cells; haemoglobinopathies affect the red blood cells; and myelodysplasia is a disorder that affects the bone marrow. Patients with such conditions can benefit from hematopoietic stem-cell transplantation (HSCT): their immune system is usually destroyed with radiation or chemotherapy, then replaced by transplanted HSCs from a donor that can reconstitute the stock of blood cells.

However, HSCT is not a simple procedure. The first step consists in collecting cells from a donor. Dr Ivanovic's work aims to make this process easier and more effective.

HSCS AND HAEMATOPOIESIS

The purpose of HSCT is to reconstitute the patient's stock of blood cells from a donor's HSCs that will proliferate and differentiate into specific blood cells.

Haematopoiesis is the process that leads to the formation of blood cells from HSCs. In a healthy adult person, it is estimated that the daily production of blood cells averages hundreds of billions units. HSCs, located in the bone marrow, are self-renewing cells: when they proliferate, some daughter cells remain HSCs while other daughter cells differentiate into a specific type of blood cell.

HSCs first give the progenitor cells. Common lymphoid progenitors then further differentiate into lymphocytes while common myeloid progenitors give red blood cells or white blood cells that are not lymphocytes.

Stem cell proliferation and differentiation are regulated by proteins named growth factors and cytokines. These molecules modulate gene expression: as they mature, cells start expressing genes and producing proteins that are specific to the type of cell they are differentiating into.

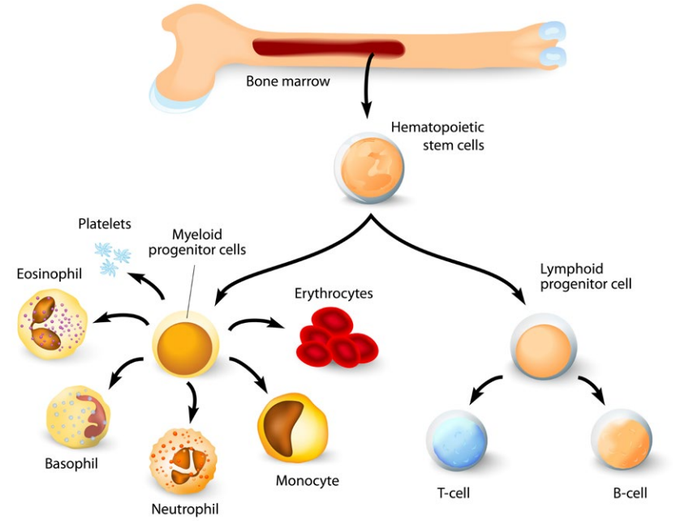
MOBILISING HSCS FROM THE BONE MARROW

HSCs can be collected from a large bone of the donor through a technique known as bone-marrow harvest. This requires local or general anaesthesia, which is not ideal.

Instead, stem cells can be mobilised from the bone marrow and collected from peripheral blood: with injections of a cytokine (a protein that sends signals to cells) named granulocyte colony-stimulating factor (GCSF), the bone marrow is stimulated and releases HSCs into the bloodstream. HSCs can then be collected through a process called cytapheresis: blood is withdrawn from the donor's arm and passed through a machine that separates and retains a specific type of blood cell while the remainder is returned to the donor's circulation.

This approach, consisting of mobilising HSCs from the bone marrow and collecting them by cytapheresis, represented a revolutionary event in hematopoietic transplantation. Instead of harvesting them from the bone marrow, mobilised peripheral blood HSCs became the most common source of stem cells for HSCT.

However, HSC mobilisation from the bone marrow is not without risks. Injection of GCSF can cause adverse events that could be avoided if HSC mobilisation was not necessary. With this in mind, Dr Ivanovic and his team studied the availability of HSCs in steady-state peripheral blood, that is to say in blood in physiological conditions or, in other words, in its natural state, without having to use mobilising agents.



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HSCS IN STEADY-STATE PERIPHERAL BLOOD

Dr Ivanovic examined the presence of HSCs in steady-state peripheral blood. Even if some reports had hinted that HSCs could be found in steady-state peripheral blood, the approach consisting of mobilising cells from the bone marrow was introduced into clinical practice, and it was so effective that strategies involving steady-state peripheral blood were abandoned.

Working at the French National Blood Service, Dr Ivanovic and his team have

Dr Ivanovic and his team studied the availability of HSCs in steady-state peripheral blood, that is to say in blood in physiological conditions or, in other words, in its natural state, without using mobilising agents.

Dr Ivanovic observed that HSCs could be recovered from leukodepletion filters (prior to transfusion) and suggested that these filters could be a source of stem cells potentially usable in cell therapy.

The team identified the optimal experimental conditions for *ex vivo* expansion of steady-state peripheral blood HSCs.

access to blood from voluntary healthy donors. Before giving a patient a transfusion, white blood cells have to be removed from the donor's blood sample. To do that, blood is passed through leukodepletion filters (LDFs). Used LDFs are discarded, but Dr Ivanovic and his team studied them and observed that HSCs could be recovered from LDFs, and therefore suggested that these filters could be a source of stem cells potentially usable in cell therapy.

This finding indicates that stem cells can be collected from peripheral blood without having to use mobilising agents such as GCSF. But the number of HSCs in steady-state peripheral blood is low and, therefore, each filter only collects a relatively low number of cells.

EX VIVO CULTURES

Faced with the challenge of the low number of cells collected by filters, Dr Ivanovic and his team further worked on these cells from steady-state peripheral blood. They found that, with *ex vivo* cultures, it was possible to amplify these cells. *Ex vivo* cultures consist of collecting cells from a living organism and culturing them in adequate experimental conditions. By doing that, Dr Ivanovic and his colleagues managed to amplify the stem cells collected from the filters: HSCs, whose frequency is low in freshly isolated cells, expand after incubation, meaning that the low number of cells increases.

In combination with ex vivo expansion, steady-state cytapheresis could become an interesting source of stem cells for hematopoietic transplantation.

IMPROVING EXPERIMENTAL CONDITIONS

Finding the ideal experimental conditions for *ex vivo* expansion of steady-state peripheral blood HSCs required some more work from the researchers. Indeed, HSCs circulating in blood and HSCs staying in bone marrow live in different environments. For example, oxygen concentration is much greater in venous blood (~13%) than in bone marrow (~3%). This impacts cell physiology and metabolism.

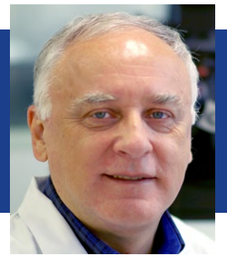
Different *in vivo* environments suggest that steady-state peripheral blood HSCs and bone marrow HSCs have different needs in *ex vivo* cultures. While much was already known about how to maintain and expand mobilised HSCs in *ex vivo* cultures, knowledge about culture of steady-state peripheral blood HSCs was more limited.

Dr Ivanovic and his team tested the effects of different parameters on these cells to identify the optimal environmental conditions and recreate them in *ex vivo* cultures. This approach was effective as they managed to maintain and expand the cells *ex vivo*. Besides, *ex vivo* culture also enhanced the individual proliferative capacity of HSCs. Finally, once injected into immunodeficient mice who cannot reject human cells, HSCs of human origin from *ex vivo* cultures can reconstitute the blood cell population in mouse bone marrow, demonstrating that they kept their stem cell ability despite the *ex vivo* expansion. This model allows to propose regenerative medicine protocols in human medicine.

STEADY-STATE CYTAPHERESIS

Dr Ivanovic's work showed that, with *ex vivo* expansion, it was possible to use steady-state peripheral blood HSCs to reconstitute the cell population. Compared to what is traditionally done in HSC transplantation, this procedure does not require mobilising HSCs from the bone marrow with agents such as GCSF.

Their results suggest that mobilisation is not required before collecting stem cells for HSCT by cytapheresis: in combination with *ex vivo* expansion, steady-state cytapheresis could become an interesting source of stem cells for hematopoietic transplantation.



Behind the Research

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Research Objectives

Zoran Ivanovic's research explores stem cell biology and transfusion technology. He conceived and developed *ex vivo* expansion procedures of stem and progenitor cells.

Detail

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Bio
Zoran Ivanovic MD, MSc, DSc, HDR, has published pioneering research into the induction of the stem cell self-renewal at low O_2 concentration. He conceived the *ex vivo* techniques for the expansion of stem and progenitors cells from cord blood and adult peripheral blood. He has published 120 articles and book chapters, and realised 160 meeting communications.

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Personal Response

Your results show that the usual mobilisation treatment is not needed to collect HSCs; do you expect to see a change in practice?

"I think that this approach could resolve the problem for individuals who are not well-suited to a GSF injection and mobilisation, or even if these procedures are inapplicable. Thus, at least for some cases the proposed approach might be an interesting alternative."