HISTORICAL BACKGROUND OF UMBILICAL STEM CELL CULTURE

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Abstract
Umbilical cord is a waste material, and therefore does not raise ethical concerns related to its use for research and medicine. Stem cells from umbilical cord have a significant advantage over cells from other sources. First, the umbilical cord is an infinite source of stem cells, because it can be taken theoretically during each delivery. Secondly, acquisition of umbilical cord is a non-invasive, safe procedure for mother and child. Thirdly, the transplantation of umbilical cord stem cells is associated with a lower risk of infection and a less-frequent "graft versus host" reaction. In this work, the authors present a historical background of research on the cell from its discovery to modern times characterized by highly advanced methods of obtaining stem cells from umbilical cord and from other sources.

Running title: History of umbilical stem cell culture

Keywords: umbilical stem cells, history of umbilical stem cells
Introduction

The construction of living organisms has been the subject of people’s interests since antiquity. In the available literature one can find information that in the years 500-428 BC, Greek philosophers were making various theories about the structure of organisms. Anaxagoras from Klazom - an ancient atomist claimed that reality is built from matter consisting of immutable and infinite “seeds” and from self-governing reason, which was supposed to set in motion and cause connection and disconnection of elements [1].

The Seventeenth to Nineteenth Centuries

In 1665, Robert Hooke, an English naturalist, observed the existence of compartments in tissues, describing the cell - the smallest functional and structural unit of living organisms capable of carrying out all life processes [2]. In 1839, two German scholars: zoologist - Theodor Schwann and botanist - Matthias Jacob Schleiden put forward a cellular theory that all living organisms are made of cells. The above theory was created in two stages. In 1838 Matthias Jacob Schleiden stated that all plant organisms were made of cells, and a year later Theodor Schwann extended the application of the German botanist by stating the existence of an animal cell [3,4]. The essence of in vitro cell culture is to maintain their vital functions outside the living organism [5]. Interest in the culture of cells, tissues and organs outside the body became the subject of interest of scientists shortly after the discovery of their existence. Carl Friedrich Wilhelm Ludwig - German physician, physiologist, professor of physiology and topographic anatomy in 1856 developed techniques of organ perfusion after removing them from the body. The essence of the experiment was to pump blood through organs taken from the body. Three years later - in 1859, a French neurologist Edmé Félix Alfred Vulpian isolated fragments of tadpole tail and attempted to breed them in water. Cells isolated by Edmé Félix Alfred Vulpian, despite their survival in water, did not reproduce. However, the experience of French neurologist showed that cell survival in vitro is possible [1]. Twenty-one years later, the English clinician Sidney Ringer conducted research on the work of the cardiac muscle of animals placed in 0.75% saline and the effect of additional substances added to it (blood, albumin, potassium). The British researcher proved that it is possible to survive the organs for a long time, provided they are kept in a sodium chloride solution with small amounts of potassium. In 1885, the German embryologist Wilhelm Roux proved that it is possible to keep the nerve plate obtained from the chicken embryo in a heated saline solution. Unfortunately, all attempts carried out resulted in a bacterial or fungal infection [6].

The Twentieth Century

In 1903, Justin Marie Jolly, a French hematologist and histologist, attempted to breed nucleated red blood cells. He was also referencing the work of the German botanist Gotlieb Haberland, who thanks to the breeding medium he developed, increased the size of the cultured cells, but did not obtain their multiplication. The blood cells cultured by Justin Jolly divided for the first 15 days, after which they gradually lost ability to cell divisions until total arrest few months after the start of cultivation [7]. In 1906, two American scientists, Charles William Beebe - zoologist and oceanographer, and oceanographer James Ewing, made a successful attempt to in vitro maintain a dog lymphosarcoma cells. The breakthrough moment in in vitro cell culture is considered to have occurred in 1907 [8]. In this year, Ross Granville Harrison, an American biologist and anatomist, achieved success in in vitro cultivation of diverse nerve cells of the radial nerve of amphibian embryos [9]. Harrison placed fragments of the nervous tissue of the frog’s embryo in a drop of congealed lymph. This experiment showed that axons of nerve cells are formed from protrusions that grow out of the body of nerve cells. Harrison also observed the movement of the cytoplasm from the cell body to the protrusions [10]. During the first observations, Harrison used frog cells, as they were not hard to maintain in cells cultures. The frog is a cold-blooded animal, so its cells do not require incubation under constant conditions. In addition, tissue regeneration is more effective in lower vertebrates. Therefore, Harrison supposed that the experience would have a greater chance of success than with the use of mammalian cells [11]. Ross Harrison is considered the creator of the methods used in cell culture [1]. The American also developed a cell culture technique “in a hanging drop”. Then, thanks to cooperation with Montrose Thomas Burrows, a surgeon and pathologist, he succeeded in developing a cell culture technique in a drop of clotted plasma placed on a microscope slide. Montrose Thomas Burrows began his collaboration with Harrison thanks to Alexis Carrel, a French surgeon, Nobel Prize winner in physiology and medicine in 1912. Burrows, as an assistant to Alexis Carrel, was sent by him to Harrison’s laboratory to learn about the cell culture techniques. Carell’s goal was to adopt Harrison’s techniques for research on warm-blooded animals. Montrose Thomas Burrows has been very successful during his cooperation with Harrison. In 1910 he successfully established a tissue culture of embryonic chicken cells [1]. After returning, Thomas Burrows and Alexis Carrel introduced a number of improvements to Harrison’s culture techniques. These improvements included, among other things, the use of a lymph instead of blood plasma and the addition of an extract from the em-
Improvements made by scientists have contributed bryo to the nutrient medium used for tissue culture. In the years 1911-1912 Carrel, in cooperation with cell biologist Georg Otto Gey, started a hanging drop. In 1933, Alexis Carrel, in continuity to learn the methods of chicken embryo tissue culture, obtained a continuous cell culture. Carrel's cell culture was continued for the next thirty-four years by Albert H. Ebeling - using it to test germicides [13]. Carrel's close associate was also Charles Augustus Linbergh - an American airman who became famous for the first lonely passage between North America and Europe. Pilot was the originator and contractor of a large number of devices used by Carrel for tissue culture. Lidbergh constructed, among others, a press for crushing chicken embryos. Carrel also worked on the construction of instruments used in the research [14].

Another breakthrough in the subject of in vitro culture took place in 1916, when Francis Peyton Rous - American physician and pathologist, Nobel laureate in 1966 with Jones for the first time use of a proteolytic enzyme - trypsin. Trypsin was used to dissociate single cells from tissue fragments. After dissociation, the cells were placed in the culture medium, resulting in a suspension of viable cells. The idea of using trypsin to dissociate individual cells is practiced to this day [15].

The 1930s was the period of further development of scientific in vitro cell culture techniques. Professor Zygmunt Grudzinski of the Jagiellonian University is considered as the forerunner of tissue culture techniques. Carrel assumed that the collection of material and the establishment and maintenance of the culture should take place in accordance with the principles of antisepsics. The place of operation should be a sterile box and the tools used should be sterile. He also assumed the use of sterile media. Alexis Carrel also introduced the method of culture in a thrombus blood clot. He was also the originator of cell culture in a coagulum formed by mixing embryo extract of chicken embryos and plasma [12]. The experience of the French Nobel Prize winner showed that, thanks to the use of an embryonic extract and asaepsis during cell cultures, it is possible to keep the cells out of the body for many years. Alexis Carrel, thanks to the regular and continuous passage of fibroblasts from the heart of the chicken to the fresh breeding medium, obtained a continuous culture. Carrel's cell culture was continued for the next thirty-four years by Albert H. Ebeling - using it to test germicides [13]. Carrel's close associate was also Charles Augustus Linbergh - an American airman who became famous for the first lonely passage between North America and Europe. Pilot was the originator and contractor of a large number of devices used by Carrel for tissue culture. Lidbergh constructed, among others, a press for crushing chicken embryos. Carrel also worked on the construction of instruments used in the research [14].

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The seventies of the twentieth century were a time of increased attempts to obtain pluripotent cells. In 1981, mouse pluripotent embryonic stem cell lines with normal karyotype were successfully isolated from blastocysts [18]. Seven years later, in 1988, on the initiative of American doctors: Hana Broxmeyer and Joanna Krutzberg and prof. Eliane Gluckman at the Paris Clinic undertook the first trial in the history of umbilical cord blood cell transplantation [19]. Umbilical cord blood is a rich source of pluripotent cells that can be dedifferentiated into any type of cell in the human body. An umbilical cord blood of a newborn sister was given to the boy who was ill with Fanconi’s anemia. A few days after the transplantation, blood cells derived from the donor cells were found in his bloodstream. The first allogeneic transplantation of frozen and banked umbilical cord blood was performed in 1993. A few years later, it was proved that the cord blood has enough stem cells to repopulate the recipient’s marrow. Evidence has also been provided that there is less risk of allograft rejection compared to bone marrow transplantation [20]. The first cord blood stem cell transplant in Poland took place in 1994 to treat a boy suffering from acute myelogenous leukemia.

The Twenty-First Century

In 2001, more than one blood unit was transplanted in the USA. In Poland, a similar transplant was performed in a patient with refractory chemotherapy-resistant acute leukemia in 2003. In March
2007, the first Polish transplant of banked cord blood was performed at the Children’s Hematology Clinic in Wroclaw. The blood for transplant was collected in 2004 [21].

Conclusion

Stem cell transplants taken from the umbilical cord over the years gain a wider range of applications and the use of cells stored in banks contributes to saving or improving the quality of life of many patients. The data of Polish Stem Cell Bank show that, since 2011, more than 1194 umbilical cord stem cell transplants were conducted in Poland [22].

Ethical approval

The conducted research is not related to either human or animal use.

Acknowledgements

This publication and its results are an outcome of a cooperation between Poznan University of Medical Sciences (Poznań, Poland) and Polish Ministry of Science and Higher Education, with Institute of Advanced Sciences Sp. z o.o. (Poznań, Poland), as a part of the “Professional PhD” programme.

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Conflict of interest statement

The authors declare they have no conflict of interest.

References
