# HUMAN TISSUE CULTURES FOR VACCINES PRODUCTION

Vladimír OLEÁR[1,2]*, Mária ŠTEFKOVIČOVÁ[1], Zuzana KRIŠTÚFKOVÁ[2]

[1] *Faculty of Healthcare, Alexander Dubček University of Trenčín, Študentská 2, 911 50 Trenčín, Slovak Republic*

[2] *Faculty of Public Health, Slovak Medical University in Bratislava, Limbová 12, 833 03 Bratislava, Slovak Republic*

*Corresponding author E-mail address: vladimirolear@gmail.com

**Abstract**

There have been arguments, particularly from religious reasons, on the ethics regarding some culture media being used in the production of traditional viral vaccines. At present, human cells (tissue and cell cultures, cell lines) can be used almost exclusively for cultivation of strictly human type viruses, including their propagation and growth. Tissue cultures that are used in the manufacture of vaccines as basic enrichment media and, for some viruses, are important due to the possibility of re-use (as opposed to the chick embryo). Where possible, animal tissue and cell cultures, such as VERO cells or canine kidney cells, are also used for the purpose of the development and production of viral vaccines. Other tissue cultures are prepared based on human cells originally derived from spontaneously or artificially aborted embryos. Human cell cultures cannot be regarded as cultures that would self-regenerate, for example, from new cells derived from fetal organs through spontaneous or artificial abortion. Cell cultures are self-reproduced and their DNA cannot be used for cloning. It should be emphasized that no abortion was carried out for the purposes of vaccine development.

A review of the literature on cell cultures used for vaccine production

**Key words:** tissue culture, cell line, vaccine

## 1 Objectives

Cell cultures are often propagated in collagen-containing media. Primary cell cultures are made up of cells taken directly from living tissues, and may contain various types of cells, such as fibroblasts, epithelial cells, and endothelial cells. For the purpose of vaccine production and research, cell cultures are designed to include only one particular strain of cells. A subculture is selected from the original cell culture, which contains only the necessary strain. Various procedures are used for the selection including centrifugation to separate large-sized and small-sized cells. Pure cell lines enable continuous monitoring, which is not easy in mixed cell cultures containing more cell types.

The longevity and reproductive ability of cells are mostly subject to Hayflick limit, named after Leonard Hayflick, who found that normal cell populations are able to reproduce only until a certain generation (50x in healthy cells) and then the cells die. However, this is not true for all cells and their cell lines, as some cell lines may be immortalized: this means that the cells were mutated to allow their reproduction for an unlimited period of time. One example is the HeLa cell line derived from tumor cells of cervical cancer in 1951 taken from a woman named Henrietta Lacks. HeLa cells, or simply HeLa, are a type of cell in an immortal cell line used in scientific research. It is the oldest and most commonly used human cell line derived from cervical cancer cells taken on February 8, 1951. The patient eventually died of cancer on October 4, 1951. This cell line was found to be remarkably resistant and fertile (cell division). Discovery of cell strains and cell lines allowed for development methods for the growth and propagation of viruses "*in vitro*" and their parallel attenuation. This is an essential requirement for the production of live vaccines, wherein attenuation of the virus is used to obtain certain properties of the vaccine virus that is unable to propagate well or even minimally in the human body. One of the procedures used for attenuation of viruses for vaccines, is subcultivation (repeated cultivation) of the virus in cell cultures at a much lower temperature than normal body

temperature. The virus adapts to the environment in order to replicate at lower temperatures, thereby losing its original ability to grow at normal body (human) temperature. The vaccine strain obtained in this manner (under strictly defined conditions, such as composition of the culture media, growth temperature, etc.) loses its infectiousness (not 100%) but yet retains its immunogenic properties [1].

## 2 Human cell culture lines

*HDC (human diploid cells):*

WI-38 (Wistar–38), human diploid lung fibroblasts, were prepared in 1961 in the US (Leonard Hayflick, Paul Moorhead) at the Wistar Institute in Philadelphia from lung cells derived from a female fetus artificially aborted in the third month of pregnancy for health reasons in Sweden and was named WI 38. Normal human cells usually divide 50 times in cell cultures and then die. Cancer cells taken from the tumor are able to divide virtually for an unlimited period of time but are not suitable for use as a culture medium. Hayflick attempted to establish a cell line that would have properties of "tumor" cells in terms of infinite division and at the same time would be safe and suitable as a culture medium. His 38th experiment with the cell line (lung fibroblasts derived from aborted fetus) was successful and Hayflick obtained cell lines that did not show any abnormalities even upon repeated cell divisions and continued to grow normally irrespective of the number of divisions (unlimited - immortal cell division). WI-38 cell lines are used e.g. during the production of rubella vaccine. The vaccine strain of the rubella vaccine, known as RA 27/3, was obtained after medically indicated therapeutic abortion from the fetus, whose mother had rubella during pregnancy. RA273 is therefore a strain of the rubella virus used for the vaccine rather than a tissue from an aborted fetus, as sometimes incorrectly described at the Internet. The viruses replicate in the WI-38 cell lines, and extracted from diploid cell culture and subsequently further processed to prepare the substrate for the vaccine. The WI-38 cell line (human lung fibroblast, normal) and the culture medium consists of an essential medium known as EMEM (Eagle's Minimum Essential Medium) with 2 mM L-glutamine, 1.5 g / L sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate and 10 % FBS (Fetal bovine Serum) [2].

*WI- 26 VA4 (Wistar institute)*

The WI-26 VA4 cell line is a derivative of the human diploid fibroblast line from embryonic lung cells derived from a Caucasian male fetus aborted in the third month of pregnancy (it is a cell line derived from the same fetus as a line WI-38, but from the 26th consecutive division) and subsequent transformation of the SV 40 virus (simian virus). The cells are positive for SV40 and neo (T) antigen. The cells contain the antigen T-Ag from the SV40 virus, which cannot be transformed to the active form of the virus [1].

*MRC-5 (Medical Research Council 5)*

In September 1966, UK scientists (J.P. Jacobs) from Medical Research Council obtained lung cells from 14 weeks old male fetus artificially aborted for psychiatric reasons from 27 years old physically healthy woman. Tissue culture thus obtained was named MRC-5. Both tissue cultures have the same number of chromosomes as normal human cells and retain the original properties for a long time; the cells are capable of 42 to 46 divisions before their death (apoptosis) [3]. The MRC-5 cell line is commonly used in the development of vaccines. MRC-5 cells, which grow adherently in fibroblast cultures, can double their population 42 to 46 times. They are suitable for the cultivation of poliovirus 1, herpes simplex virus, and vesicular stomatitis virus (Indiana strain) [4]. Culture medium: Ham's F12 medium (1: 1 mix), enriched with 2 mM L-glutamine and 5% fetal bovine serum [5].

*IMR-90 (Institute for Medical Research)*

The new human diploid cell line IMR-90 was designed for research and technological purposes. National Institute on Aging (NIA), National Institutes of Health (NIH), based on contract with the Institute for Medical Research (IMR) in Camden, New Jersey, started developing the IMR-90 line as a substitute for the WI-38 line. The culture was collected from a female human fetus at 16 weeks of age (length 7 cm) obtained by therapeutic abortion on July 07, 1975 from 38 year old Caucasian woman. The culture medium for the IMR90 cell line is EMEM (Eagle's Minimum Essential Medium) + 500 ml of 2 mM Glutamine + 10% fetal bovine serum. It can be sub-cultured 16 times [6].

*PER.C6:*

This cell line was prepared for the first time at the request of the pharmaceutical company Eye Integrated pharma (Greenville). The name originated from the word "Percivia," which combines the name of the first phase of technology (PERC) and the word "VIA," which consists of Latin VI = 6, and symbolically "VIA" also means the path or life. The cell line derived from retinal tissue was taken by Dr. Van Der Eb (Leiden University) from a fetus aborted at 18 weeks gestational age. In 1985, it was made into a cell line that has been used since 1995. Abortion was artificial at the request of the mother (social reasons) with otherwise normal pregnancy.

The PER.C6 cell line is derived from human embryonic retinal cells transformed with type 5 adenovirus (AD5) E1A and E1b genes. The PER.C6 line is sometimes compared with the HEK 293 line, but the reasons for their preparation have been different. The purpose of the HEK 293 line was basic research, while the reason for the preparation of the PER.C6 line was pharmaceutical production of adenoviral vectors for vaccines [7]. It is a growth medium suitable for a wide range of human viruses causing diseases, and is used for the production of inactivated, whole, live attenuated, live vector, split, subunit, or recombinant vaccines. It is suitable for culturing viruses for the manufacture of antiviral vaccines, such as: Ebola, West Nile fever, influenza, malaria, or MTB [8].

*HEK 293 cells: Human embryonic kidney cells*

HEK 293 cells were obtained by transformation of human embryonic kidney cells with modified DNA with adenovirus 5 in Alex Van der Ebo's laboratory in Leiden in the Netherlands and were first described in 1977 [9]. HEK cells were obtained from embryonic kidney cells of a healthy fetus spontaneously aborted in 1972. Frank Graham carried out the transformation with adenoviral DNA, and was successful at the 293th attempt, which reflects in the name of the cell culture HEK 293. This cell line is currently one of the most commonly used in the manufacture of viral vaccines. The use of HDC (Human Diploid cells) for the production of viral vaccines allowed the development of vaccines against rubella, hepatitis A, varicella, and new vaccines against rabies. The two basic cell cultures (WI-38 and MRC- 5) have been maintained in laboratory conditions for over 50 years.

*Namalwa*

This human B-lymphocytes line was derived primarily from an African child with Burkitt lymphoma in 1967. It is used mainly for the manufacture of interferon. The cells grow as tumors in nude mice (immunodeficient mice) [10]. Long-term culturing of Namalwa cells led to the presence of complex chromosomal rearrangements, including a large number of unidentified marker chromosomes. The cells secrete low levels of monoclonal antibodies (IgM, lambda light chain) of unknown specificity. Namalwa cells were used as a source of lymphoblastoid interferons and are used as an alternative to other mammalian tissue systems. Namalwa cells can be grown in large amounts in a chemically defined serum-free medium, which allows large-scale industrial production [11].

## 3 Conclusions

The World Health Organization [12] maintains control over all cell lines used in practical life and for experimental purposes. WHO RCB (Reference Cell Banks), was founded in 1987 and is intended for a wide range of tests to examine the suitability of cell lines for their use in the manufacture of vaccines. RCB WHO provides a unique source for the development of future biopharmaceuticals, which requires a cell substrate with a safe and reliable history of use. RCB is also accepted as a bank to generate parent line cells. Cell cultures used for production of the vaccines must by in SPC of vaccine [13].

RCB ensures and provides the essential conditions for the development of vaccines worldwide, which include:

- continuity of the lines from the original cells and derived cell lines and materials used in the preparation of secondary lines,
- ability to open international scientific research and cooperation for technical examination of the properties of cells and presence of foreign microorganisms,
- the results and characterizations have been reviewed and published,
- examinations evaluated and confirmed by WHO expert opinion and qualified as suitable for use in the manufacture of vaccines,
- supply of cells without any restrictions regarding the intellectual property rights to the final products, and
- the only source of cells with increasing and scientifically and technically updated test data security and safe history of use, with growing confidence of the manufacturers, regulatory authorities and public policy makers.

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## REFERENCES

[1] History of Vaccines by Wistar Institute President, dostupné na: http://www.historyofvaccines.org/content/blog/history-vaccines-wistar-institute-president

[2] WI38 (human lung fibroblast cell line) lysate (ab3960) - Abcam; dostupné na: http://www.abcam.com/wi38-human-lung-fibroblast-cell-line-whole-cell-lysate-ab3960.htm)

[3] MRC-5 ATCC ® CCL-171™ Homo sapiens lung Normal, dostupné na: http://www.atcc.org/products/all/CCL171.aspx#characteristics

[4] Human Fetal Lung Fibroblast Cells (MRC-5 Line), dostupné na: http://micro.magnet.fsu.edu/primer/techniques/fluorescence/gallery/cells/mrc5/mrc5cells.html

[5] MRC-5 - CLS Cells Line service, dostupné na: http://www.cell-lines-service.de/content/e3969/e4567/e4610/index_eng.html

[6] IMR-90 ATCC ® CCL-186™ Homo sapiens lung normal, dostupné na: http://www.atcc.org/products/all/CCL-186.aspx#generalinformation)

[7] Vaccines and related biological products advisory committee meeting wednesday, may16, 2001, dostupné na: http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3750t1_01.pdf

[8] Med-Immune Inc. To Produce New Flu Vaccine Using Cancer-Potential Aborted Fetal Cell Line PER C6, dostupné na: http://www.cogforlife.org/fluPress.htm

[9] Graham FL, Smiley J, Russell WC, Nairn R. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J Gen Virol 1977; 36 (1): 59-74.

[10] http://www.copewithcytokines.de/cope.cgi?key=Namalwa

[11] Henault M, Lee LN, Evans GF, Zuckerman SH. The human Burkitt lymphoma cell line Namalwa represents a homogenous cell system characterized by high levels of Toll-like

receptor 9 and activation by CpG oligonucleotides. J Immunol Methods 2005; 300 (1-2): 93-9.

[12] WHO Reference Cell Banks (RCBs) dostupné na: http://www.who.int/biologicals/areas/vaccines/WHO_reference_cell_banks/en

[13] Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Whitehouse Station, N.J., U.S.A. table lists selected preparations for use in the prevention of infectious diseases by immunization. (2006) dostupné na: http://issuu.com/thelifesciencenews/docs/vaccines