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The electroporation system for cells mixture monitored by morphometric analysis of the hybrid picture

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The outline of issues connected with contemporary application of monoclone antibodies and techniques for obtaining hybrids was presented in the study. The results of research concerning modifications of Zimmerman's methods were obtained. They prove higher efficacy and selectivity of the solution proposed. The system of electroporation with changed geometry of electrodes and parameters of current is being proposed. The procedure is controlled entirely by microprocessor, from the stage of technology parameters' control in incubators of initial cells to creation of appointed mixture of cells in a mixer, and then division into portions, pumping the mixture into hybridisation chamber and, after hybridisation, pumping the mixture over into separate sections of incubator. The hybridisation system was modified. Transparent water coat was constructed. It was connected to thermostat, on which transparent hybridisation chamber was installed. Lighting from underneath and gap lighting of chamber enable continuous observation of suspended cells by means of microscope lens being connected by picture channel to computer. The software analyses the picture with respect to hybrid selection. The cells marked are planimetrically analysed during the time which was programmed. When the morphometric criteria are met suspended cells are pumped over to separate sections of incubator, where selective breeding of hybrids is carried out. Selection of hybrids takes place in electroosmosis gradient under morphometric control of cells in microcapillary systems.

Key words: modification of electroporation process, survey hybridisation chamber, morphometric and dynamic analysis of hybrids, computerised system for the control of hybridisation

1. Introduction

One of the crucial problems connected with modern laboratory diagnostics and systems for selective control of medicine therapy are monoclone antibodies. They

constitute a practical key to immunological and chemical peculiarity and present unique possibility of finding selectively in organisms shield cells or even single chemical individuals. A single and simplest antibody is tetramer composed of two polypeptide heavy chains and two light chains connected by means of sulfhydryl bridges. The structure consists of invariable area characteristic of a given immunoglobin class and variable area comprising ends of four chains, and their three-dimensional conformation and amino acid sequence determine nearly complete molecular peculiarity. Configuration of functional groups and unbalanced chemical bonds, as well as molecule vector decomposition of magnetic and electric fields, all in the form of three-dimensional mosaic, are so unique that they determine peculiarity of molecule bond with compatible chemical structure. At the same time differentiation of chemical compounds takes place, not only on the basis of their molecular formula but also taking into account their three-dimensional conformation, including stereoisometry [20], [21].

A specific molecule identifier enables diagnostics owing to its linking with substances for radioactive, spin, fluorescent or classic dye marking. There are also interesting therapeutic possibilities based on interconnecting it with cytostatic, antibiotic, or any drug, whose performance should be very selective, and its too high concentration in circulating blood causes specific side effects in other organs [1], [2], [4], [5], [7]–[11], [14]–[19]. Oncology seems to be particularly important area of its application.

- In biotechnology, the precursor substances of monoclone antibodies are plasmocytes – specialised cells of immunological system, whose natural function is to take

a selected antigen standard, e.g. from macrophages, and production of antibodies compatible with its antigen determinants.

- In the next step, an attention is drawn to the occurrence of pathological plasmacytes showing uncontrolled tendency to produce antibodies (plasmacytoma). Such plasmacytes are found in some types of growing diseases.
- Basic biological structure which is able to produce monoclone antibodies are heterogenic cell hybrids (atypical cells). They do not originate in mitotic and meiotic cycles, but as a result of merging two initial cells, often of utterly different types. Because of numerous protection mechanisms such a phenomenon occurs very rarely and spontaneously in natural conditions.

It was observed that such a change might be induced by viruses, multihydroxide alcohol, ionising radiation or electromagnetic fields. The above information was practically used for designing systems allowing us to control hybridisation with use of:

- Controlled infection of appointed mixture of selective cell cultures, for example with Sendai viruses.
- Treating the mixture of selective cell cultures with detergents which diminish surface tension of cell membrane (e.g. multihydroxide alcohol).

• Exposing the mixture of selective cell cultures to a directed influence of electromagnetic field and electric discharge (Zimmerman's method) [12], [13], [23], [25].

Nowadays there exist many commercial laboratory systems which enable controlled hybridisation. Unfortunately, the efficacy of obtaining living and dividable hybrids is relatively low in terms of the amount of cells which are put in for hybridisation and hybrids impaired and unable to divide any further.

2. The aim of the study

In order to design a system, we need:

• computerised parameterisation of hybridisation process in terms of cell culture and dosage,

• controlling the proportion of the suspended matter being mixed,

• control and selection of hybridisation parameters,

• continuous controlling the image of hybrid-forming dynamics.

3. Structure and characteristic of the system

3.1. Laboratory position

The laboratory part of the system is presented in figure 1. It consists of an inspection chamber [19] situated under the microscope lens, from which, by means of the picture channel composed of "Hiton" camera and "Fly video", the converter card images of the preparations of hybridised cells are inserted into author's morphometric software.

Inspection chamber consists of thermostat blanket on which hybridisation chamber is situated. Hybridisation chamber differs from traditional one because of electrode geometric deformation [13]. The change in design causes greater predictability and efficacy of the hybridisation process because of specific guidance of line of electric field force, while cell cords are being formed and needle potential appears [23].

Hybridisation chamber is connected to mixer chamber and into the latter the appointed amounts of cells are inserted from any number of thermostats by means of programmable peristaltic pumps. On filling the hybridisation chamber with suspended matters of cells in specific proportions and on conducting electroporation controlled by author's operating software, the cells are observed and evaluated by author's morphometric software, which monitors dynamics of their growth. If the sample obtained fulfils morphometric criteria, the contents of the chamber is pumped into suitable incubator which enables further in vitro culture [18], [19].

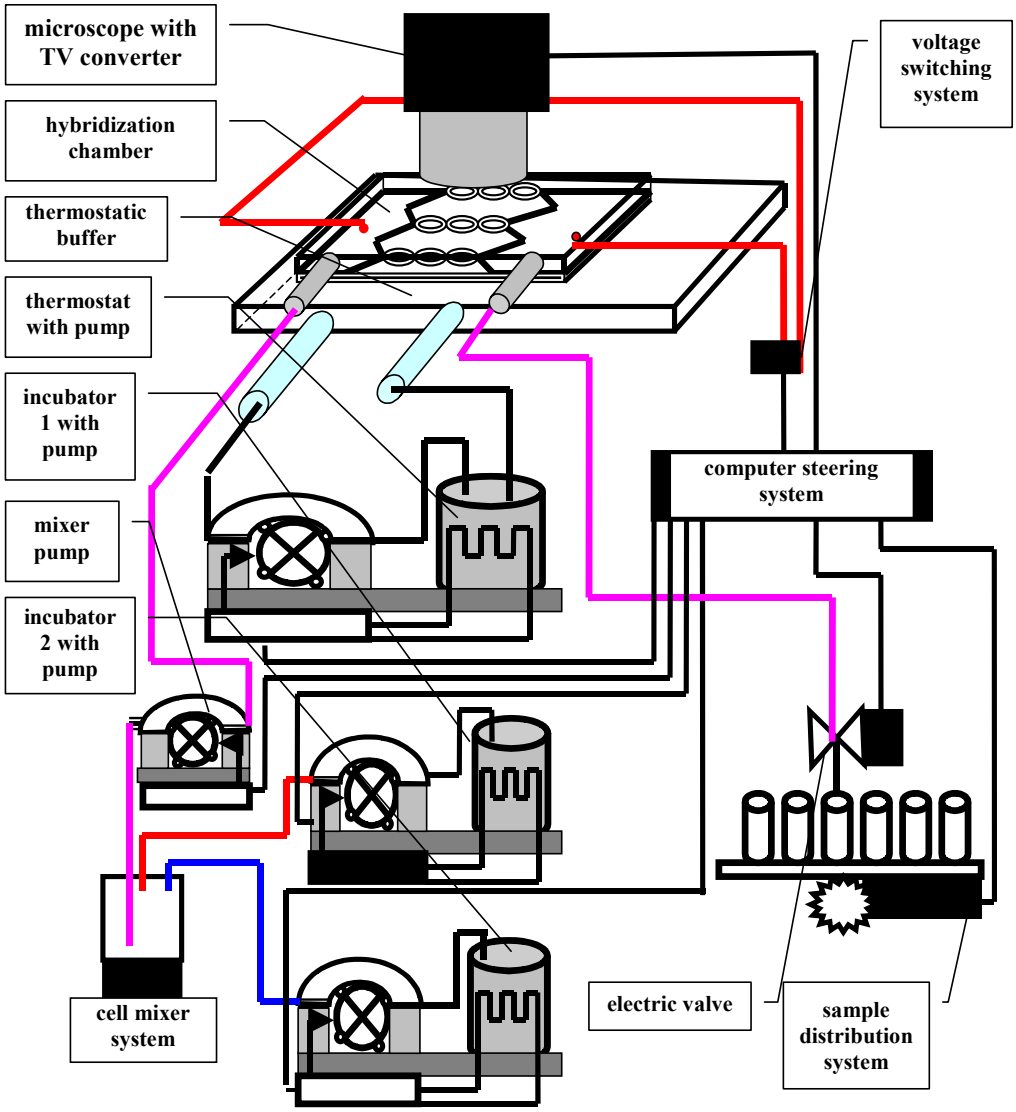


Fig. 1. Block diagram of the laboratory position for cell hybridisation

3.2. Control of the hybridisation process

For a control of laboratory position, 89C52 processor was used together with mulitiplexor and drivers for specific functions of the system presented in figure 2. Tool functions are coded in programmable memory 32kB; there is an additional RS232 link for bi-directional communication with a PC. The control of specific modules is presented in detail in figure 3.

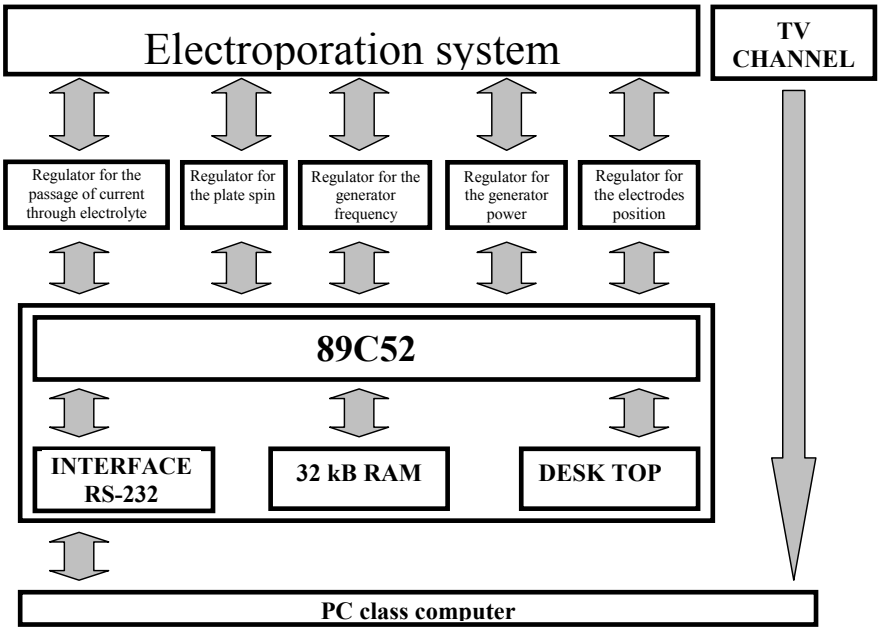


Fig. 2. Electroporation system controlling

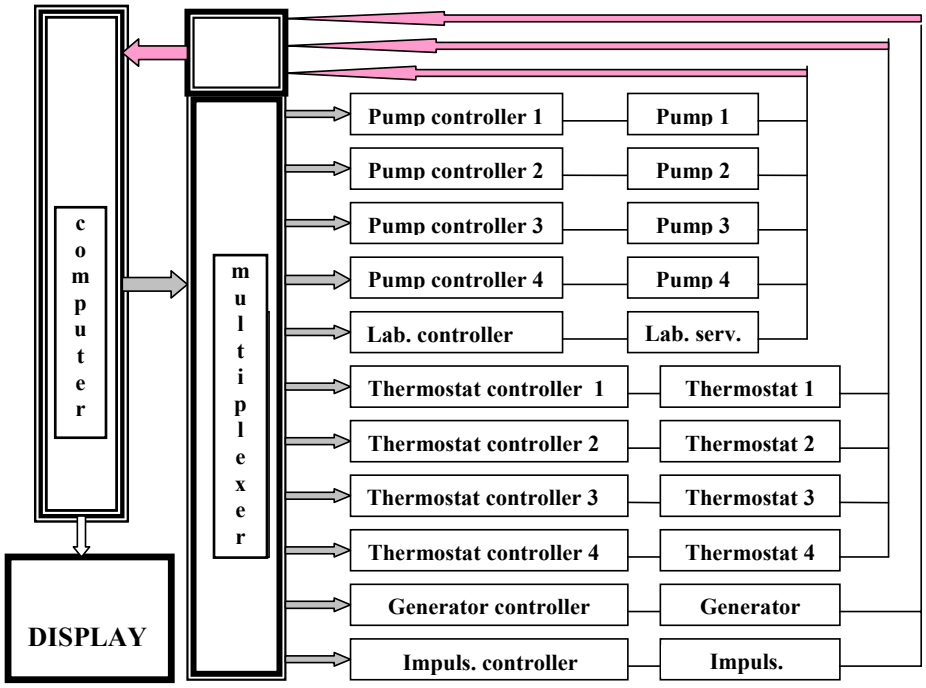


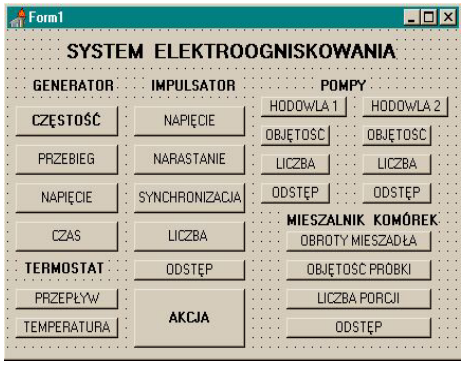
Fig. 3. Block diagram of the controller of parameters of hybridisation process by means of modified method of electrofocusing

3.3. Controlling software

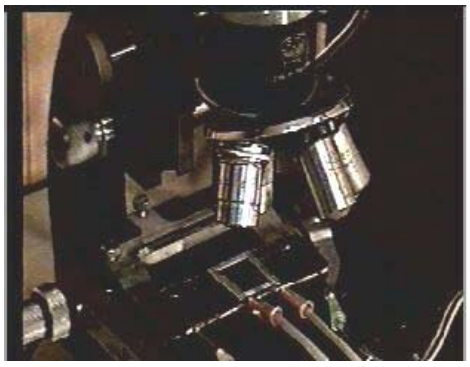
The software managing the system consists of two parts:

• Electroporation module written in "Delphi 2.0" which enables the loading of voltage, intensity, frequency and passage of current time in hybridisation chamber parameters, parameters steering the thermostat functions in a given culture, pumps proportioning the flow of suspended matters of cell and cell mixer (figure 4a). The program co-operates with experimental chamber (figure 4b) in order to create optimal hybridisation parameters for a given hybrid type, and then, on the basis of the parameters obtained, can control the parameters of technological process of antibodies production using big hybridisation flow chamber (figure 5).

• Morphometric module written in "C+" which enables putting microscope images from hybridisation chamber in storage (figure 4b) continuously or optionally changeable. The program enables marking the chosen cell structures by adequate cursor, planimetric measuring of the cell marked in specific time intervals and generating
a diagram of changes in cell area as a function of time (figure 5). The program enables also creation of cell standard, which qualifies the hybrid obtained for further culture.



a) software operating interface



b) experimental electroporation chamber

c) electroporation chamber for industry

Fig. 4. Interface for system operator

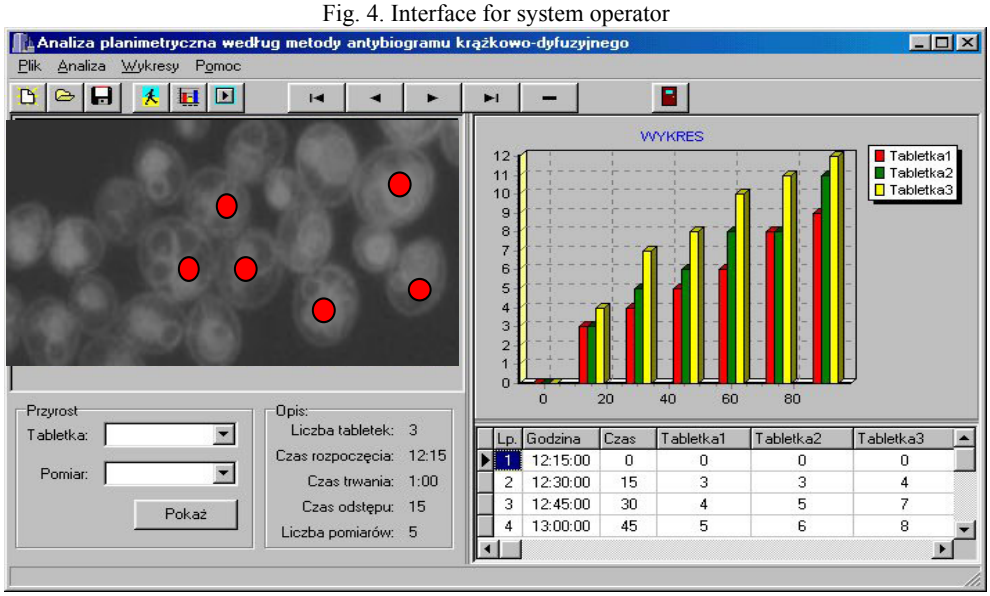


Fig. 5. Morphometric window

4. Report

The laboratory position presented is a modern system which combines practical advantages of many interesting biotechnological solutions, adding to their possibilities its own and original technical solutions. The system is a quick link between demand, created by the market and production potential. It enables easy specification of biotechnological parameters in production process of any kind of hybrid. This is possible due to transformation of the parameters of experimental chamber into the parameters of technological chamber. By means of computer-controlled hybridisation steering systems and storing cell images the interactive and quickly accessible data base systems can be created. The system may be very useful in long-term production programs. But it is cardiology [3], [6], endocrinology [4], [21] and oncology which seem to be particularly interesting implementation area. The idea of hybrid creation on the basis of tumour removal during operation and then production of peculiar to a specific patient monoclone antibodies brings huge possibilities to improve therapy by means of:

- interconnecting the antibodies obtained with cytostatic and then obtaining their high therapeutic concentration during possible metastasis but with considerably lower exposure of remaining patient's organs [10], [11], [22], [24], [28],
- hybrid selection taking into account cytotoxic antibodies' production, which can be applied during the second batch on concluding chemotherapy [26], [27], [29]–[32].

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