

## CELL GROWTH SYNCHRONIZER

Device producing synchronized, adherently growing cell lines – completion of the development phase of the commercial application PCT/CZ2010/000043

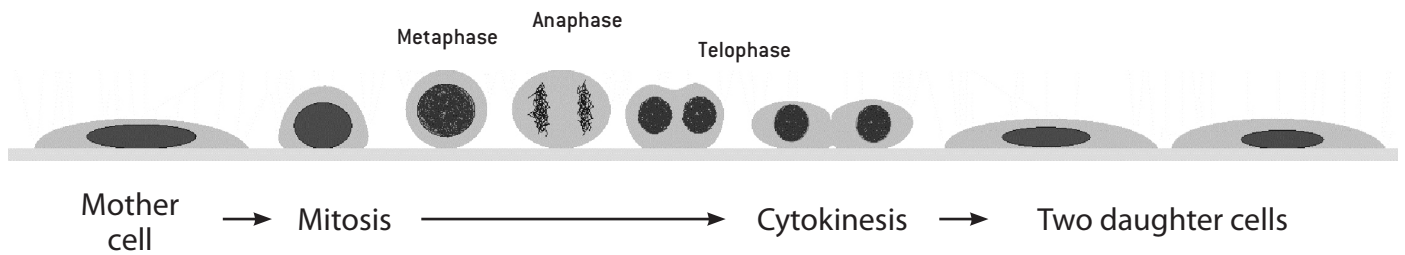
Synchronized growth induction of cell population cultivated in-vitro

This method was discovered, perfected and tested in 2009.

The primary use of the presented technology is to stop the cell growth in a specific phase of its growth cycle. This is achieved by using a specialized piece of equipment. The conception of the device equipped by its own high-power battery allows the usage in standard cell incubators and is fully compatible with standard tissue culture plastic. The device consists of a special vibration unit which causes a defined vibration deflection of a freely suspended platform. This vibration causes a movement of the culture medium in a culture bottle which is firmly attached to the platform with elastic straps and forces are applied to the cells growing attached to the bottom of the culture flask. Cells with weak adhesion to the bottom are then being released into suspension. These are mostly mitotic cells, which are physiologically incapable of full adhesion to the bottom because of their mitotic state.

Mitotic cells which have been released into suspension are prevented from adhering again by the constant vibration and remain in suspension. This induced change stops the mitosis of the mitotic cells in their late telophase. In other words, the mother cell will not completely divide into two daughter cells (see Fig.1). This inhibition of proliferation is non-toxic and fully reversible within the subsequent 24 hours. If the inhibited telophase cell is allowed to adhere to the bottom of the culture bottle, it finishes the mitosis and continues to grow. Such suspension of telophase-captured cells created from a normal exponentially growing population by this instrument is therefore an ideal basis for a new cell population which for experimental reasons needs to be homogeneously synchronous in the terms of their cell cycle phase.

### Normal growth



### Application of the device

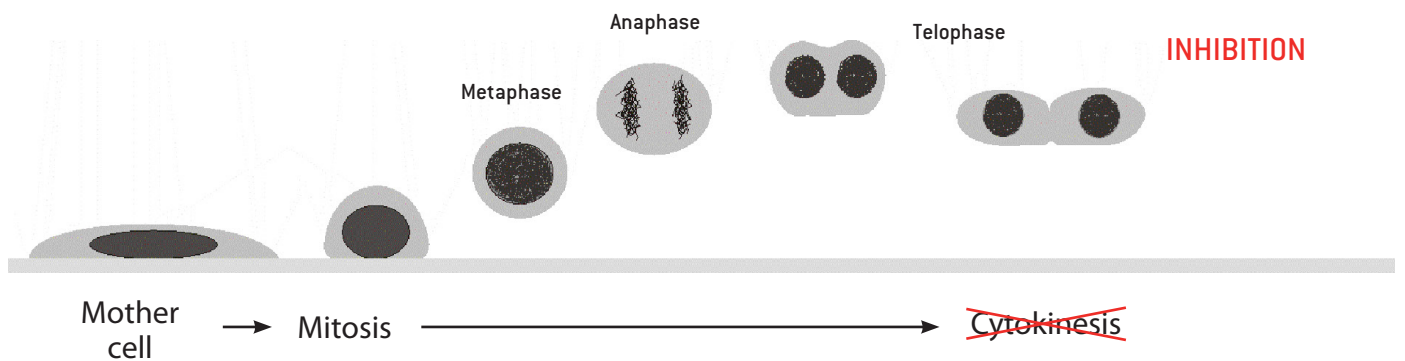


Figure 1: Principle of cell growth inhibition in telophase

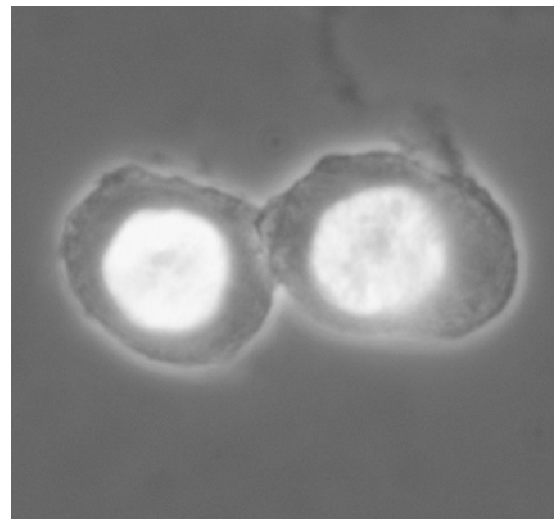
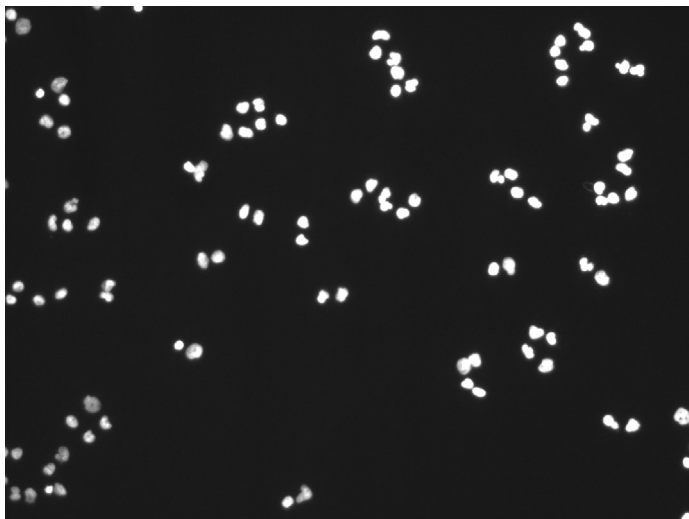
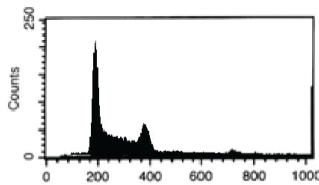
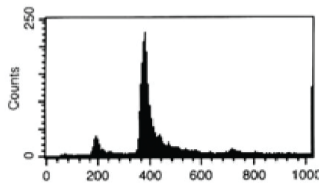


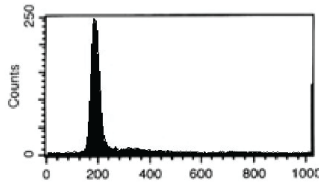
Figure 2: Micrograph showing the binuclearity (telophase) of the cells of the suspension fraction.



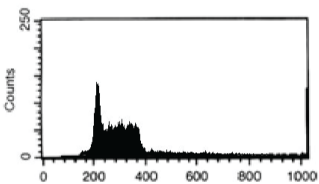
i. Normal flow-cytometry population profile (based on DNA content) of the exponentially growing human cell line (U-2-OS) cultivated in-vitro.



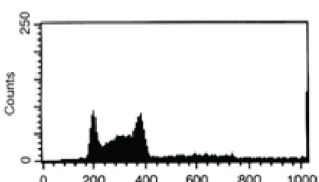
ii. Fraction of suspension cells obtained from the device (vibrations applied for 24 hours). The majority of the obtained synchronous population consists of binuclear cells (see Fig. 2) which form a substantial peak in the „400“ section of the profile.



iii. 2 hours after reseeding the suspension fraction obtained in step 2. The cells have finished mitosis and are progressing through next G1 phase or entering the DNA replication phase (S-phase).



iv. 10 hours after reseeding the suspension fraction, the majority of the cells are in the S-phase.

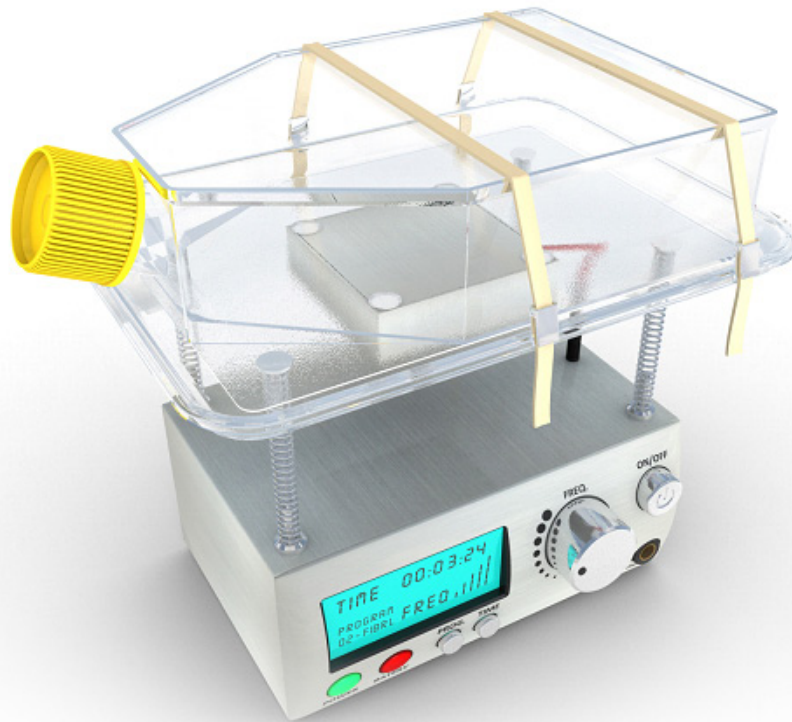


v. 16 hours after reseeding the suspension fraction. Most of the cells are finishing the S-phase and are entering mitosis (M-phase).

**Figure 3: Cell cycle profile analysis of a cell population initiated from synchronized suspension fraction**

Both the method and the device are protected under PCT/CZ2010/000043. A working prototype is available which has proved the practical feasibility of the solution and its efficiency. The instrument consists of three main parts:

- freely suspended platform with brackets for attaching culture bottles (the culture bottle is not a part of the device and not under the patent protection)
- vibration unit firmly attached to the platform
- base with battery supply and vibration unit regulation



*Figure 4: Possible design of the instrument*

### **Another possible application of the synchronizer: automatic chemical feeder for in-vitro cell cultures:**

Apart from its primary function (see above), the instrument has another possible application which increases its overall applicability in laboratory work. Once again, the periodical vibration deflection of a freely suspended platform is used. This causes movement of a culture medium within a culture bottle firmly attached to the platform with elastic straps, which apply force to a labile cartridge from inert material containing chemical additives. As a result of this force, the cartridge is capsized and the chemical additive comes in contact with the culture medium. Furthermore, the ongoing vibrations will cause a perfect mixing of the additive and the culture medium. Combined with a timer which can be programmed with starting time and duration of vibration, the instrument can batch the chemical without any laboratory assistant present, and at a predefined point in time. It is a very valuable application allowing to “supply” to the cells’ required substance(s) according to the design of the particular study, and regardless of the laboratory assistant’s working hours. This function partially substitutes some expensive robotic systems and is included in a calculation in a study by Trystom company (study available upon request).

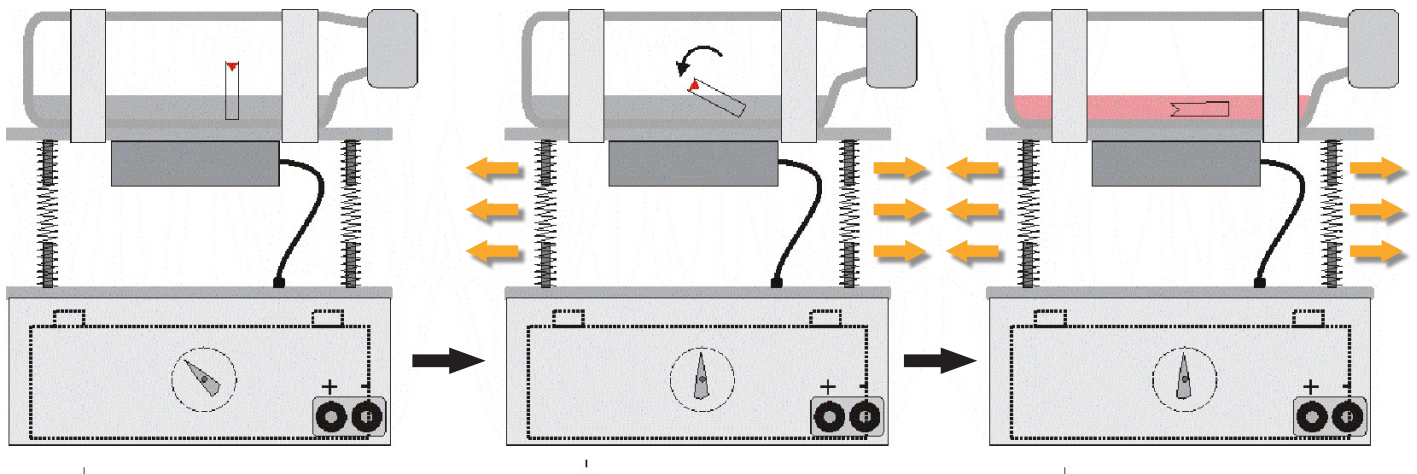


Figure 5: Application of the instrument as an automatic chemical feeder for the in-vitro cell cultures

### Expected costs – production preparations:

Expected costs preceding the serial production (based on the analysis of construction and production costs performed by Trystom, spol. s r.o.):

- 1) costs for resolving the mechanical construction and electronic module  
– approximately **EUR 3300**
- 2.) costs for manufacturing a new prototype and preliminary analysis  
– approximately **EUR 1200**
- 3.) costs for the validation of the instrument, including the exercise tests, endurance and resistance tests, electric safety and electromagnetic compatibility tests, ES declaration and CE certificate – approximately **EUR 3400 – 4800**



### Expected serial production costs:

Synchronizer will consist of: battery, small engine, vibration plate with elastic mounting, waterproof control unit and engine case, control panel, display, controls, rechargeable module, charger. The instrument will be packed in a paper box with foam transport padding and supplied after a 1-hour "burn-in operation" with a warranty of 24 months.

### Estimated prime cost:

| Pieces per year | Shipment | Price per piece |
|-----------------|----------|-----------------|
| 10 – 100pcs     | 10pcs    | EUR 380*        |
| 100 – 1000pcs   | 100pcs   | EUR 270*        |
| 1000 – 10000pcs | 1000pcs  | EUR 100*        |

\* on condition: manufactured in PRC

All prices exclude VAT.

### Estimated selling price:

Estimated selling price considering products of similar construction requirements **EUR 1200 – 2000.**

### Customers:

Laboratories of basic and applied research, and biotech companies using human and/or animal cell lines cultivated *in-vitro*.

### Expected sales:

Tens of thousands pieces worldwide.

### Sales calculation:

The calculation is based on the sales estimate for the Czech Republic performed by three independent centers which meet a condition of being a potential end customer. This number was applied to the total number of technical patents in the corresponding class for the Czech Republic and other countries.



Number of patents in class c12m3/00 c12m3/02 c12n5/02  
(in which the presented technology is classified)

| Country        | Number of patents | Estimated sales             |
|----------------|-------------------|-----------------------------|
| Czech Republic | 18                | tens of pieces              |
| Germany        | 1289              | thousands of pieces         |
| Japan          | 4369              | thousands of pieces         |
| USA            | 7045              | tens of thousands of pieces |

Advantages as compared with competition:

| Method              | Generality | Toxicity | Yield  | Population purity | Application requirements | Costs |
|---------------------|------------|----------|--------|-------------------|--------------------------|-------|
| Chemical inhibition | High       | High     | High   | Medium            | High                     | Low   |
| Serum insufficiency | Low        | Medium   | High   | High              | Low                      | Low   |
| Elutriation         | High       | Low      | High   | Low               | High                     | High  |
| Cell sorting        | High       | Medium   | Medium | High              | High                     | High  |
| Mitosis shake-out   | High       | Low      | Low    | High              | Medium                   | Low   |
| PCT 2010/000043     | High       | Low      | High   | High              | Low                      | Low   |

Chart: Advantages and disadvantages of standard synchronization methods compared to the presented technology (green = positives, red = negatives)

Rival product does not exist. No other company offers the same or similar product.

### Summary of the key features of the technical solution:

- new method for producing synchronized, adherently growing cell lines without the use of any toxic chemicals
- low costs device
- no special bulk material needed, common culture bottles are used
- possibility of other applications (automatic chemical feeder, universal battery-powered shaker)

### Conditions for granting the license:

Conditions for granting the license will be discussed in person.

### Contacts:

Univerzita Palackého v Olomouci, Křížkovského 8, 771 47 Olomouc, Česká republika

IČ: 619 89 592    DIČ: CZ61989592

#### **Professor Jiri Bartek, M.D., Ph.D.**

Co-inventor of the device and method  
Danish Cancer Society, Copenhagen, DK  
email: [jb@cancer.dk](mailto:jb@cancer.dk)  
phone: +45 35 25 73 57

#### **Martin Mistrík, Ph.D.**

Co-inventor of the device and method  
University of Palacký in Olomouc, CZ  
email: [martin.mistik@upol.cz](mailto:martin.mistik@upol.cz)  
phone: +420 585 634 873

#### **Martin Šimo**

Project Manager  
The Science and Technology park, University of Palacký in Olomouc, CZ  
email: [martin.simo@vtpup.cz](mailto:martin.simo@vtpup.cz)  
phone: +420 585 631 438