The influence of the centrifugation temperature on urological carcinoma cell lines

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Introduction

Until now centrifugation at room temperature (21 °C) has been considered the standard process in cell culture.

However, with regard to cell biology, this temperature frequently does not correspond to optimal culture conditions.

With human cell lines in particular, centrifugation at 37 °C, the core temperature of the human body, may have a positive effect on the viability of the centrifuged cells, reducing cell damage.

For this reason, the influence of the centrifugation temperature on the viability of the cells was examined using different cell lines of urological tumors.

Materials and methods

Cell lines
Studies were carried out on different human tumor types of the T24, HTB44 and HTB J82 cell lines, also referred to below as tumor entities.

The T24 human continual cell line equates to a papilloma or well differentiated bladder carcinoma.

This cell line has a monolayer growth form and the morphology of the cells is epithelial.

The HTB J82 urethral cell line equates to a urethral carcinoma of the bladder. This cell line also has a monolayer growth form; the cell morphology is epithelial.

The HTB 44 human cell line equates to a renal carcinoma.

The growth form is monolayer and the cell morphology epithelial. The *in vitro* cytopathology corresponds to a papillary renal adenocarcinoma.

Prior to centrifugation, the cell lines were in the subconfluent growth phase.

The cells were tested for viability using Trypan blue staining. This method detects the degree to which the cell membrane is permeated by the stain.

Healthy, viable cells do not absorb the stain whereas damaged cells do, appearing blue under the microscope.

The viability studies were carried out to determine the proportion of viable to non-viable cells.

The proportion of non-viable cells, i.e. dead cells, may be increased as a result of forces experienced during centrifugation.

The cell count required for the evaluation of the study was carried out in the Neubauer Chamber.

Centrifugation
Centrifugation of the three cell lines was carried out at the current standard temperature of 21 °C, and at 37 °C to determine the comparison value.

What was being examined was the hypothesis that centrifugation at 37 °C leads to significantly less cell damage. This is determined using the ratio of non-viable : viable cells. The results are given as a percentage of non-viable cells.

The following equipment and methods were used for cell centrifugation:

Eppendorf Centrifuge 5702 RH, at 1,200 rpm (this corresponds to 220 x *g*), for 3 min at 37 °C.

Centrifuge Eppendorf 5702 R, at 1,200 rpm (220 x *g*), for 3 min at room temperature (21 °C in this case).

The A-4-38 swing-bucket rotor with adapters for 50 ml conical centrifuge tubes was used in both centrifuges.

Results

On average, the studies found an increase of 5-6 % in the number of non-viable cells after centrifugation at room temperature for the three T24, J82 and HTB44 cell lines.

The number of cells damaged or traumatized by centrifugation was reduced when centrifugation was carried out at 37 °C, human core temperature.

The individual results for the cell lines are presented as follows:

T24 cell line
Centrifugation at room temperature increased the percentage of damaged cells by a factor of 3.1, while centrifugation at 37 °C increased the proportion of non-viable cells by a factor of just 2.

The direct comparison of room temperature (21 °C in this case) and 37 °C led to the following result:

After centrifugation at room temperature, the proportion of non-viable cells was on average 6 % greater than with centrifugation at 37 °C (see also Table 1).

HTB J82 cell line
With the HTB J82 cell line, centrifugation at 37 °C detected, on average, more than 5 % fewer non-viable cells.

In comparison to the culture prior to centrifugation, the number of cells damaged by centrifugation at room temperature was increased by a factor of 2.2. Centrifugation at 37 °C saw a significantly lower increase at a factor of 1.7 (see also Table 2).

HTB44 cell line
With the HBT44 renal carcinoma cell line, 5 % fewer non-viable cells were detected when centrifugation was carried out at 37 °C.

In comparison to the culture prior to centrifugation, the number of damaged cells was increased by a factor of 4.0 as a result of centrifugation at room temperature.

After centrifugation at 37 °C, the factor was significantly lower at 2.4 (data not shown).

Date	Cell line	Percentage of non-viable cells prior to centrifugation [%]	Percentage of non-viable cells after centrifugation [%]	
		RT	RT	37 °C
14.01.2004	T24	8	18	8
21.01.2004	T24	2	12	6
28.01.2004	T24	6	20	18
Average value:	T24	5.3	16.7	10.7
RT: Room temperature, 21 °C in this case				

Table 1: Results of the cell count measurement of the well-differentiated T24 bladder papilloma cell line before and after centrifugation with specification of the percentage of non-viable cells.

Date	Cell line	Percentage of non-viable cells prior to centrifugation [%]	Percentage of non-viable cells after centrifugation [%]	
		RT	RT	37 °C
02.02.2004	J82	6	12	10
16.02.2004	J82	10	22	16
19.02.2004	J82	18	38	34
24.02.2004	J82	24	36	30
26.02.2004	J82	8	24	16
02.03.2004	J82	12	28	24
10.03.2004	J82	4	16	14
16.03.2004	J82	14	28	20
24.03.2004	J82	6	18	10
31.03.2004	J82	2	8	6
07.04.2004	J82	10	20	14
14.04.2004	J82	10	22	14
Average value:	J82	10.3	22.7	17.3
RT: Room temperature, 21 °C in this case				

Table 2: Results of the cell count measurement of the poorly differentiated J82 bladder carcinoma cell line before and after centrifugation with specification of the percentage of non-viable cells.

Conclusion

Centrifugation at 37 °C is favorable for both the bladder and renal carcinoma cell lines studied. There is an identical effect for the different cell lines which can be assigned to other tumor entities.

The practical conclusion is therefore that, compared to standard centrifugation, centrifugation at 37 °C results in significantly less cell damage and replaces the previous standard method.

The 37 °C centrifugation methods appears to be valuable or not to be discounted for a number of experiments in the field of cell biology.

Centrifuge 5702 RH with active temperature control

Eppendorf offers the Centrifuge 5702 RH for temperature-precise centrifugation at 4 °C and 37 °C.

This centrifuge has a cooling system and additional active heating system up to 42 °C, enabling an optimal temperature of 37 °C to be precisely maintained for the samples.

An active heating system means that the centrifuge can be heated via the integrated heating without turning of the rotor.

The Centrifuge 5702 RH can therefore also be kept ready for use in so-called standby mode at 37 °C for the duration of the entire working day up to 8 hours.

IVD Directive

The Centrifuge 5702 RH complies with the regulations of the IVD (In Vitro Diagnostics) Directive.

The IVD Directive regulates medical products which supply information on *in vitro* studies carried out on samples taken from the human body (including physiological or pathological conditions or anomalies) or which help to monitor therapeutic measures (IVD Directive, Art. 1).

The IVD Directive and the associated standards (EN standards) have established a uniform quality and safety level for these products across Europe.

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