THE USE OF BULL SPERMATOZOA SUSPENSION FOR EVALUATION CYTOTOXICITY

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INTRODUCTION

Required for all types of medical devices, cellular toxicity testing is described in ISO 10993.5-96: «Tests for Cytotoxicity-In Vitro Methods». This standard presents a number of test methods designed to evaluate the acute adverse biological effects of extractables from medical device materials. These test methods use constant cell lines and have some disadvantages such as long-term operations, necessity to guarantee sterile conditions for cells and impossibility to test non-sterile extracts. Prime culture of bull spermatozoa has not been widely used in assays of this type but it permitted us to develop very precise, quick and cheap assay.

METHOD

Bull spermatozoa suspension is a cheap and accessible biological material which is easy to produce thanks to well developed processes of artificial fertilization and readily availiable from the rejected hereditary-inadequate stock. It has standard cell concentration and constant sensibility. Frozen cells stored in liquid nitrogen in Dewar vessel are used that allows to ensure the unlimited storage and reduce time for work preparation. When you use bull spermatozoa suspension there is no need to subculture cells in sterile coditions to produce multiple large flasks of cells. The motility of spermatozoa suspension m is used as the endpoint. Motility is proportional to the moving spermatozoa concentration c_m and average modulus of the cell velocity v, $m = c_m v$. Cell concentration in samples is about $8 \cdot 10^5$ ml^{-3}. The spermatozoa suspension motility measurement is done strait in suspension. Samples are placed in disposable glass flat capillaries of 25 mcl volume and of 200 micron depth which are applied as chambers. This way of measurement permits not to grow near confluence cell monolayers and save not less than 24 hours. As soon as the extract is ready it takes 10 minutes to defrost granule of the bull's frozen sperm in glucose-citrate media, prepare reference and examined samples and start the experiment. Duration of the experiment is 3 hours. Short duration of expirement permits to test non-steril extracts. The method has no requirements for sterilyty of materials, instruments and euqipment used. The motility of spermatozoa suspension is measured by specially developed cytotoxicity analyser (*see Fig. 1*). The operating principle is based on real time automatic computer microscopic videoimage analysis of spermatozoa suspension to measure its motility. The values of bull spermatozoa suspension motility for reference and examined samples are measured in equal intervals and accumulated, till motility becomes close to zero. The analyzer simultaneously fulfils function of incubator maintaining 37°C during the entire experiment. Scoring for cytotoxicity is based on the value of the toxicity index I_t which is equal to the ratio of the weighted average time of spermatozoa suspension motility in the examined sample to that of spermatozoa suspension motility in the reference sample. I_t value is expressed in percents. Toxicity index is calculated automaticaly. The value of I_t less than 70% shows that extract is cytotoxic.

CONCLUSIONS

- Prime culture of bull spermatozoa is adequate for cytotoxicity assay.
- Results of tests using bull spermatozoa suspension are identical to those using culture cells.
- Results are obtained within 3 hours and less.
- The method permits to test non-steril extracts.
- The method is easy-to-use in accordance with ISO 10993.5-96. Besides, it is useful for screening various products.

RESULTS

Bull spermatozoa and other culture cells have the same sensibility. Evaluation of inhibiting concentrations IC50 for a few compounds using bull spermatozoa and mouse fibroblast (NIH 3T3) gives close results (*see Table 1*). Correlation coefficient R=0.75. Cytotoxicity evaluation according to ISO 10993.5-96 for medical devices using bull spermatozoa and mouse fibroblast (NIH 3T3) gives the same results. Medical gloves were tested to demonstrate facilities of the method (see *Table 2*).

Validation of the test method showed that test method has rather nice reproducibility. Variation coefficient of I_t is less than 10%.

Table 1: Inhibiting concentrations IC_{50}

№	Compound	IC_{50} mmol/l	
		Bull spermatozoa	Mouse fibroblast*
1	Methanol	790	930
2	Ethanol	560	379
3	Acetone	510	444
4	Cyclohexanone	12,3	26,3
5	Phenol	9,55	3,01
6	Formaldehyde	0,04	0,12

*Values IC_{50} are taken from The Registry of Cytotoxicity: Testing in Cell Cultures To Predict Acute Toxicity (LD50) and Reduce Testing in Animals.

Table 2: Cytotoxicity test results according to ISO 10993.5-96 for medical gloves

№	Brand	Result	
		Bull spermatozoa	Mouse fibroblast
1	MediGrip PF	fail	fail
2	MediGrip Plus	fail	fail
3	Encore Orthopaedic	fail	fail
4	Microthin Nutex	fail	fail
5	Exam Tex Plus	fail	fail
6	Micro-Touch HydraCare	fail	fail
7	Dermaclean	fail	fail
8	Profeel DHD Polyisoprene	fail	fail
9	Gammex PF HydraSoft	fail	fail
10	Ultrafree Max	pass	pass
11	Protegrity Blue with Neu-Thera	pass	pass
12	Protegrity MicroSMT	pass	pass

Fig. 1: Cytooxicity analyzer