Biocompatibility Testing of Medical Devices using Assay Ready Cells

For medical devices or any material meant to come in contact with a patient, the testing of biocompatibility according to ISO 10993 guideline is required to obtain regulatory approval of the product. Here we demonstrate that the testing can be reliably performed with assay ready L-929 cells which are used instantly from a frozen vial without prior cultivation. The assay ready cells which are provided in a validated kit adhere quickly after thawing and display an unaltered sensitivity to extracts from reference materials compared to cells which have maintained in culture before use.

introduction

Medical devices must be reviewed for their proper function as well as for the compatibility of the device with the surrounding tissue. One aspect of this biocompatibility testing is to determine the cytotoxicity of an extract prepared from the device's material as laid down in part 5 of the ISO 10993 guideline. Immortalized mammalian cells are incubated in vitro with the extracts for a certain time and cell viability is determined by using dyes like MTT or XTT which are metabolized in living cells.

Commonly, these tests are conducted with cells which have been maintained in culture for several passages to ensure optimal cell fitness. However, in recent years it has been demonstrated for various applications that cryopreserved cells can be used instantly after thawing when optimized freezing protocols were applied. These Assay Ready Cells (ARC's) immediately regain their full function and fitness and do not undergo an extended recovery or lag-phase after resuscitation.

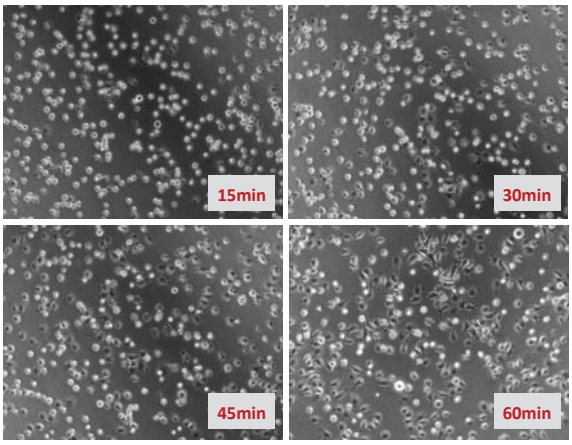

Fig 1: Adherence of AssayReady L-929 Cells after thawing

preparation of assay ready cells

To prepare assay ready cells from L-929, a murine fibroblast cell line recommended by the guideline, the cells were expanded under optimal culture conditions and kept in exponential growth phase. The cells were plated at 20.000 cells/cm^2 in RPMI 1640 supplemented with 10% FBS and 2mM L-Glutamine at 37°C in a humidified atmosphere with 5% CO_2. For further large-scale expansion in T-flasks and CellSTACKs the cells were gently detached with Accutase before becoming confluent.

For harvesting the cells were detached, washed once in culture medium and were resuspended in medium containing 5% DMSO for cryoprotection. The cells were automatically dispensed into 1.8ml cryovials at 3 million cells/vial using an XSD-Biofill decapping and filling device. Finally, the cells were cryopreserved in a Cryomed 7452 controlled rate freezer at a cooling rate of 1°C per minute before transferred to storage in the vapor phase of liquid nitrogen.

preparation of reference extracts

Reference materials (RM) provided by the Food and Drug Safety Center of the Hatano Research Institute (Japan) have been used to prepare extracts according to ISO 10993 part 12 (Tab. 1).

Reference Material	
RM-A moderate toxicity	Polyurethan film containing 0.1 % zinc diethyldithiocarbamate (ZDEC)
RM-B weak toxicity	Polyurethan film containing 0.25% zinc dibuthyldithiocarbamate (ZDBC)
RM-C non-toxic control	High density polyethylene film

Tab 1: Reference Materials

Ten pieces each of an equivalent of 6cm^2 were extracted in 2ml assay buffer (RPMI, 10% FBS, 2mM L-Gln) for 24h at 37°C in an incubation shaker.

thawing of assay ready cells

One vial of assay ready L-929 cells was thawed for 2 minutes in a water bath at 37°C and the content was transferred into 8ml prewarmed RPMI and centrifuged at 80xg for 3 minutes. The cells were resuspended in 10ml assay medium (RPMI 1640, 10% FBS, 2mM L-Gln). An aliquot of the suspension was used to determine cell count and viability parameters in a CASY TT. Instantly after thawing viability was at 98%, amount of debris was below 13% and aggregation (1,2) as low as during normal cell cultivation.

EUROPEAN OFFICE

+49 (160) 987 577 56

acCELLerate GmbH
Osterfeldstraße 12-14
22529 Hamburg - Germany

please@accellerate.me

www.accellerate.me

US OFFICE

+1 (732) 698 3404

acCELLerate GmbH
1 Jill Court, Bldg. 16/10
Hillsborough, NJ 08844 - USA

To investigate the kinetics of adherence after thawing, the cells were seeded into a 96-well plate and cultivated at 37°C. Every 15 minutes the adherence of the cells were analyzed by phase contrast microscopy. The assay ready L-929 cells adhere very quickly and were fully attached one hour after seeding. The morphology of the cells look healthy and homogeneous similar to cells from a continuous maintenance culture (Fig. 1).

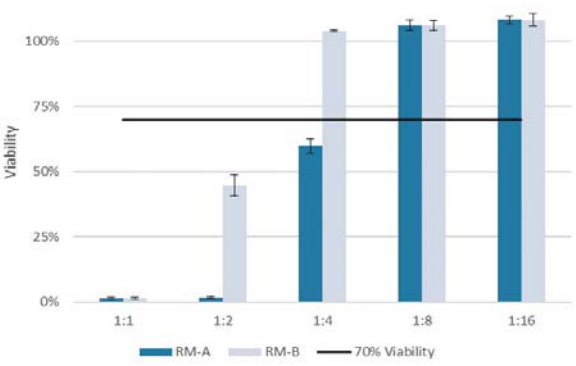

Fig 3: Viability of assay ready L-929 cells after treatment with reference extracts.

biocompatibility testing

To compare ARC's with cells from a continuously passaged culture one vial of a regular L-929 cell bank was thawed and passaged three times according to the recommended cultivation protocol. These cells and assay ready cells prepared as described above were seeded into a 96-well plate at 30.000 cells/well. The cells were incubated at 37°C for 1h to let them attach to the well surface. After adherence of the cells, the supernatant was removed and 100µl of serial dilutions prepared from the extracts were added to each well. The cells were incubated for 24h at 37°C before 50µl of XTT staining solution (XTT 1mg/ml in RPMI 1640 supplemented with 25µM PMS) were added to each well. After 4h, the absorption of the metabolized formazan was measured at 480nm against 690nm for reference in a Safire 2 plate reader (Fig. 2).

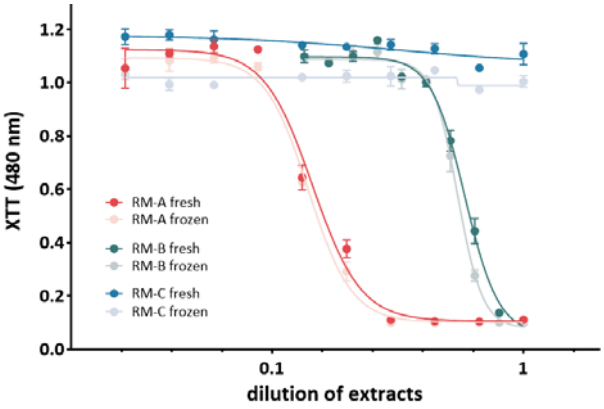

Fig 2: Dose dependent toxicity of reference extracts tested on fresh versus assay ready frozen L-929 cells.

	Fresh Cells		ARC's	
RM-A	IC50: Slope: Top: Bottom:	0.150 -4.54 1.124 0,106	IC50: Slope: Top: Bottom:	0.139 -5.04 1.093 0,101
RM-B	IC50: Slope: Top: Bottom:	0.583 -6.49 1.097 0,056	IC50: Slope: Top: Bottom:	0.546 -8.72 1.087 0,078

Tab 2: Parameters of dose-response-curves

While non-toxic control extract was as expected not cytotoxic to the cells, RM-A (moderately toxic) and RM-B (weakly toxic) displayed a dose-dependent reduction in XTT metabolization indicating a does dependent toxicity of the extracts. The results obtained with assay ready cells and cells from a continuously passaged culture were very comparable (Tab. 2).

For further validation the testing was confirmed according to the ISO 10993. 100µl of the cell suspension corresponding to 30.000 cells were dispensed into a 96-well plate. After attachment the supernatant was replaced by 100µl of 2-fold serial dilutions prepared from the reference extracts. Viability was determined by XTT as described above. According to the guideline a material is considered to be cytotoxic if the test extract reduces the viability of the cells below 70% compared to the control cells. RM-A is cytotoxic already at a dilution of 1:4 while RM-B has a cytotoxic effect only at a 1:2 dilution of the concentrated extract (Fig.3).

discussion

Assay ready L-929 cells provide a robust tool to test biocompatibility of medical devices according to ISO 10993-5. Instantly after thawing the cells display the same sensitivity to extracts prepared from toxic reference material as cells from a continuously passaged maintenance culture. By using assay ready cells, prepared in large homogenous batches, the cell-dependent variability of biocompatibility testing can be reduced which increase the reliability of the assay. Because assay ready cells are instantly available from a frozen stock, biocompatibility testing can be performed at a higher flexibility without significant lead time for the pre-cultivation of the cells.

related products

instaCELL® biocompatibility assay kit I
validated kit with assay & extraction buffer, reference materials, XTT viability assay reagent, 96-well assay plates, and assay ready frozen L-929 cells (CatN° Sf160)

L-929 assay ready cells (CatN° RE772)

XTT viability assay (CatN° RX758)