APPLICATION NOTE No. 470

Gentle for the Earth and Cells: Comparison of Cytotoxicity Parameters of Eppendorf Tubes® BioBased and standard Eppendorf Tubes®

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Abstract

With increasing environmental awareness and thus more stringent requirements imposed on life science laboratories, lab plastics present a growing challenge with respect to sustainability. One of the key approaches to improve sustainability of lab plastics is to use recycled and renewable feedstocks in their production.
In this study, a potential material cytotoxicity of the Eppendorf Tubes® 50 mL made of an ISCC PLUS certified polypropylene bio-based material was assessed in comparison to standard fossil-based Eppendorf Tubes® 50 mL. The cytotoxicity effects of both material types were comprehensively evaluated by cell morphology and viability assays with compliance with the ISO 10993-5: 2009 and ISO 10993-12: 2012 standards. Neither fossil-based nor bio-based material induced any major morphological changes or caused cell viability attenuation. This indicates that bio-based material offers excellent properties regarding cell culture parameters, which are identical to fossil-based material.

Introduction

Conical tubes with screw cap belong to the most used laboratory vessel formats and are universally applied in a variety of laboratory procedures. With the recent increase in environmental awareness, also in the life science laboratory, lab plastics present a growing burden with respect to sustainability. One of the key approaches to improve sustainable properties of plastics in the lab is to use recycled and renewable feedstocks in their production.

For the first time, Eppendorf is able to offer a generation of tubes in 5.0 mL, 15 mL, 25 mL and 50 mL formats that are made of an ISCC PLUS (International Sustainability & Carbon Certification) certified polypropylene based on renewable reused raw materials [1, 2] applying the mass balance approach.
The objective of this study was to assess a potential material cytotoxicity of Eppendorf Tubes® 50 mL made of bio-based material in comparison to their standard fossil-based counterparts. The cytotoxicity effect was evaluated qualitatively (cell morphology evaluation) and quantitatively (MTT assay). The extraction conditions for the tube material (37 °C for 72 hours and 50 °C for 24 hours) and all test procedures were compliant with the ISO 10993-5: 2009 ("Tests for in vitro cytotoxicity") and ISO 10993-12: 2012 ("Sample preparation and reference materials") standards.

Materials and Methods

Materials

The following polypropylene conical tubes 50 mL were evaluated:

> Eppendorf Tubes® BioBased 50 mL, Sterile, (order no. 0030 122 542)
> Eppendorf Tubes® 50 mL, Sterile, (order no. 0030 122 178)

Methods

Preparation of Liquid Extracts

Controls and all tubes tested (bio-based and standard) were first decontaminated with isopropanol 70% and handled aseptically during all subsequent steps. Materials were cut into small pieces, then placed into the extraction vessels with a normalized amount of extraction liquid: completed medium (MEM, glutamine 4 mM, penicillin 100 UI/mL, streptomycin 100 µg/mL, FBS 10%). The extraction conditions for the tube material are described in the above mentioned ISO standards and were: 37 °C for 72 hours and 50 °C for 24 hours. The extracts were used for cell culture immediately after the end of the incubation time. Each experiment was performed in triplicates.

Cell Culture

L929 cells were cultured in the complete medium (ATCC, 30-2003) (5% CO_2), then digested by Trypsin/EDTA 25% to get a single cell suspension. After inactivation of Trypsin-EDTA, cells were centrifuged and diluted in fresh culture medium to obtain a $1x10^5$ cells/mL suspension.

Cell Morphology Evaluation

After cell cultivation for 48 hours, the cell morphology was first examined under microscope. The morphological changes (e.g., detachment, cell lysis, vacuolization, etc.) were assessed. For each condition, the morphological status of cells was graded according to the table 1 shown below. A grade greater than 2 is considered as a cytotoxic effect.

Grade	Reactivity	Conditions of all cultures
0	None	Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

Table 1: Qualitative morphological grading (ISO 10993-5)

Cell Viability – MTT Assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) is cleaved to formazan dye by cellular enzymes, which is directly correlated to the number of metabolically active cells in the culture.
After the morphology assessment, the culture medium was discarded and replaced by 50 µL/well of freshly prepared MTT solution (1 mg/mL). Cell culture plates were incubated with the MTT reagent for 2 hours (37 °C, 5% CO_2), shaken for 1 minute and placed in the spectrophotometer for a readout at 570 nm with a reference reading at 650 nm. The measured absorbance was directly correlated to the number of viable cells as follows:

$$\text{Viab. \%} = \frac{100 \times OD_{570e}}{OD_{570b}}$$

OD_{570e} is the mean value of the measured optical density of the test sample.
OD_{570b} is the mean value of the measured optical density of the blank.

A reduction of viability below 70% (compared to the control sample) was considered as a cytotoxic effect.

Results and Discussion

Cell Morphology Evaluation
After cultivation of cells for 48 hours in presence of the tube material extracts, the L929 cell culture morphology was examined under microscope and the morphological status of cells was graded according to the table 1 classification (see methods). As shown in figure 1 and figure 2, for all conditions, the cell morphology was not affected, when cells were cultured in the presence of material extracts. Neither fossil-based nor bio-based material induced any major morphological changes under the extraction conditions tested: 72 hours at 37 °C or 24 hours at 50 °C.

	72 h at 37 °C	
	Eppendorf fossil-based	Eppendorf bio-based
Repl. 1	1	0
Repl. 2	0	0
Repl. 3	0	0

	24 h at 50 °C	
	Eppendorf fossil-based	Eppendorf bio-based
Repl. 1	0	0
Repl. 2	0	0
Repl. 3	0	0

Figure 1: Cell morphology grading of the L929 cell culture depending on the applied extraction condition and the extracted sample

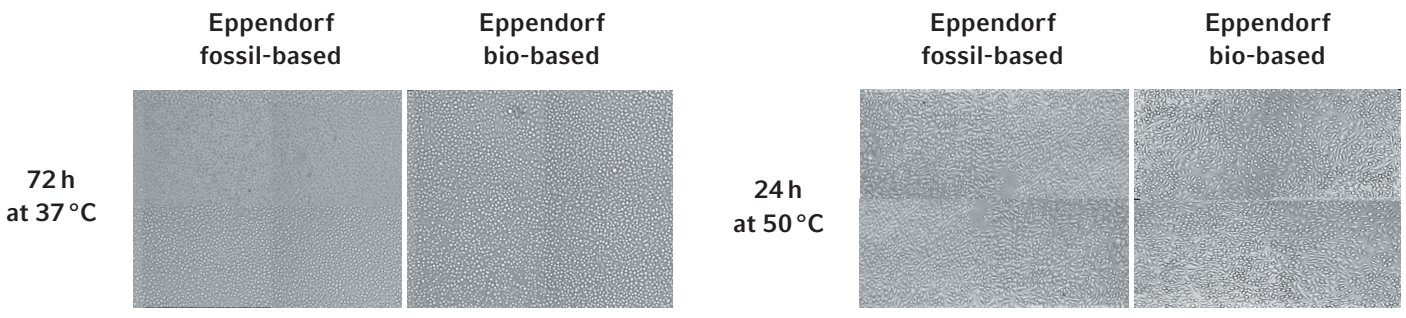

Figure 2: Images of the L929 cell culture depending on the applied extraction condition and the extracted sample

Cell Viability MTT Assay

After the morphology assessment, the cell viability was quantified by an MTT assay which is indicative of living cell metabolism. The percentage of viable cells was assessed by comparison to the controls. According to the ISO 10993-5 standard, cell viability of below 70% indicates a cytotoxic effect. The quantification evaluation confirmed unambiguously cell morphology observations. As shown in figure 3, the mean cell viability value is distinctly above 70% regardless of the extraction condition applied or the tube material under evaluation. The slight decrease in cell viability observed with long-term extracts (72 hours at 37 °C) may be indicative of unspecific degradation of cell medium (FBS).

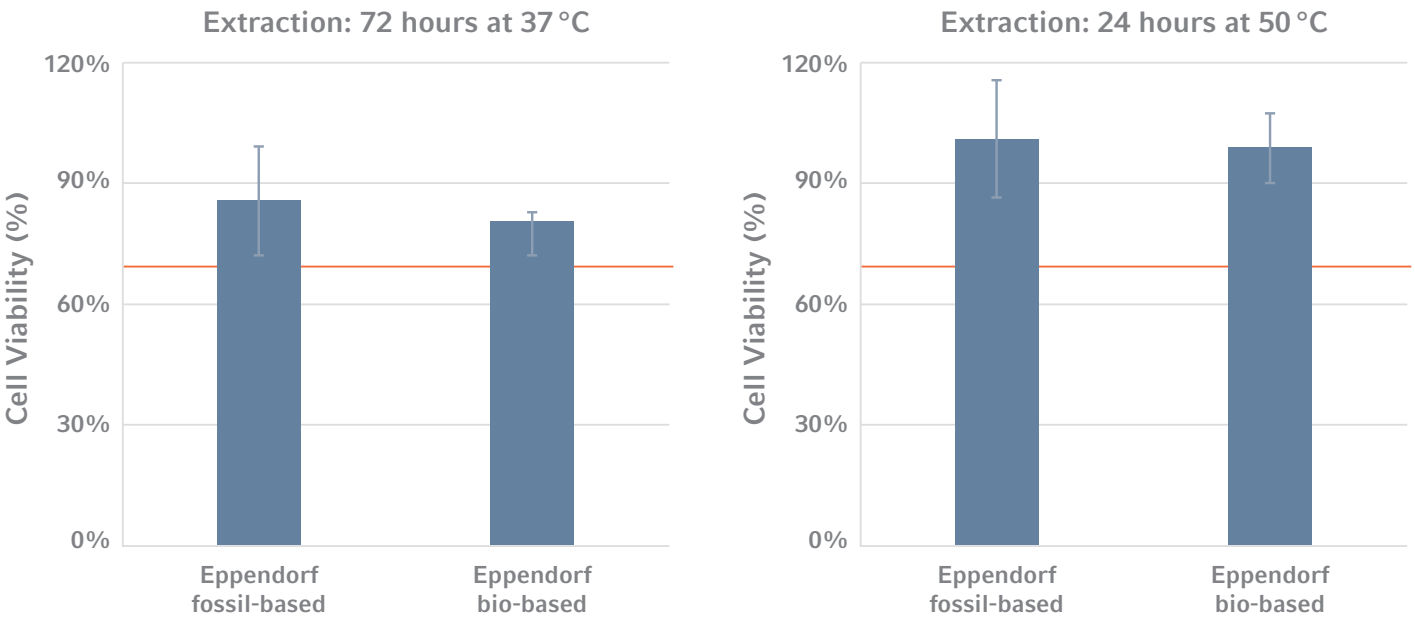

Figure 3: Cell viability of L929 cells depending on the extraction condition and the extracted tube material

Conclusion

In this Application Note we assessed a potential material cytotoxicity of the Eppendorf Tubes® BioBased 50 mL made of an ISCC PLUS certified polypropylene bio-based material in comparison to standard fossil-based Eppendorf Tubes® 50 mL. The cytotoxicity effects were evaluated with compliance with the ISO 10993-5: 2009 ("Tests for in vitro cytotoxicity") and ISO 10993-12: 2012 ("Sample preparation and reference materials") standards and included a comprehensive cell morphology and cell viability evaluation.

Neither fossil-based nor bio-based material induced any major morphological changes or cell viability attenuation. This indicates that bio-based material offers excellent properties in respect to cell culture parameters that are identical to the properties of fossil-based material. Bio-based consumables therefore present a major development in improving overall lab consumables sustainability while maintaining the same product quality and performance.

Literature

[1] For more information on the ISCC system please visit: www.iscc-system.org
[2] Hermuth-Kleinschmidt K., Consumables Made of Bioplastics Enter the Lab, Eppendorf White Paper No. 78, Eppendorf

Ordering Information

Ordering information

Description	Order no.
Eppendorf Tubes® BioBased screw cap, sterile, 5 mL	0030 122 518
Eppendorf Tubes® BioBased screw cap, sterile, 15 mL	0030 122 526
Eppendorf Tubes® BioBased screw cap, sterile, 25 mL	0030 122 534
Eppendorf Tubes® BioBased screw cap, sterile, 50 mL	0030 122 542

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