Extractables and Leachables in Gene and Cell Therapies

The assessment of extractables from single-use systems

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An evaluation is presented, demonstrating evidence that process equipment related leachables (PERLs) from single-use systems (SUS) pose low risk for gene and cell therapy applications. It is shown that available SUS extractables data can serve as a basis for the extractables and leachables assessment in cell and gene therapy (CGT) applications. However, exposure estimation and toxicological evaluation tools need improvement. Thus, a modeling concept was developed to calculate PERLs in the liquid phase and adsorbed on the therapeutic cells under dynamic process conditions. The model revealed PERL exposure levels to patients and cells which were significantly lower compared to those obtained by conventional assessment methods. Additionally, the effect of PERLs on human cells were screened with a high-throughput cell-painting assay using a U2OS human cell line. It was shown, that out of a range of 45 commonly found extractables only 4 compounds influenced any of the 579 cell features analyzed. Since modeled PERL exposures to cell-based medical products is low and because SUS are sufficiently biologically inert, they are suitable as production environment for therapeutic cells.

1. Introduction

The following article summarizes a presentation from the ATMP-track of the GMP-Pharma Congress held on the 29th of Mar 2023 at the Rhein-Main-Congress-Center in Wiesbaden (Germany). It summarizes challenges and highlights possible solutions to dedicated questions associated with an extractables and leachables assessment in the cell and gene therapy area.

Cell and gene therapy (CGT) products offer new treatment options and enormous hope for patients around the world. Yet along with this promise come new and unique challenges for the qualification of the products and the respective production processes. The evaluation of process equipment-related leachables (PERLs) from single-use systems (SUS) is one example, where some uncertainties exist on how to properly qualify the process equipment. [1,2] Unequivocal authorities demand extractables and leachables (E&L) assessment of materials in the manufacturing stream contacting a drug substance and drug product. [3,4,5] The methodologies on extractables testing and assessment for SUS in biopharmaceutical production, e.g. for the manufacturing of protein-based products like mAbs, are well developed.[6,7,8,9] However, several differences between CGT and traditional biopharmaceutical manufacturing requires to challenge and revise the methods commonly used for safety assessments of extractables and PERLs. [10]

CGT manufacturing relies heavily on SU devices and assemblies due to the high flexibility of use, ensuring at the same time product sterility and safety. [11] From sample collection to cell expansion and patient delivery, CGT drug products contact a variety of SU devices and assemblies at virtually every stage. These include bioprocess storage and media bags, filters, bioreactors, connectors, tubing and fittings. [1] In addition, the trend towards smaller, fully enclosed systems – for example SU isolators – to produce drug products for personalized treatments (autologous therapy) means that surface-to-volume ratios are relatively high compared to traditional biopharmaceutical drug production. This increases the chance, that PERLs, migrated out of the SUS, have an impact on the process performance, the product quality, or the patient health.

In cell-based *ex vivo* gene therapies (fig. 1), the cells themselves are the product. Since cells are living entities, PERLs that accumulate in the liquid phase or on the cells themselves can negatively impact cell characteristics and viability, as well as its critical quality attributes (CQA). Eventually resulting in lower product yields, reduced therapeutic

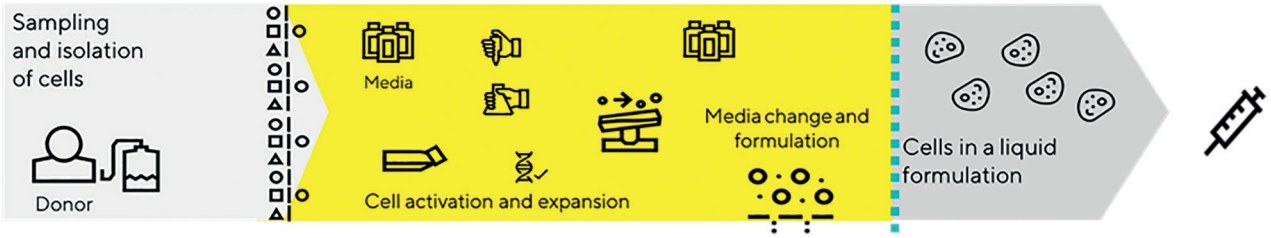

Figure 1: In cell-based CGT, donor cells are taken, isolated, manipulated ex vivo and administered back to the patients. Sampling, ex-vivo manipulation and application of the product rely on disposable SUS but lacking a dedicated downstream purification (all figures provided by the authors / Sartorius Stedim Biotech).

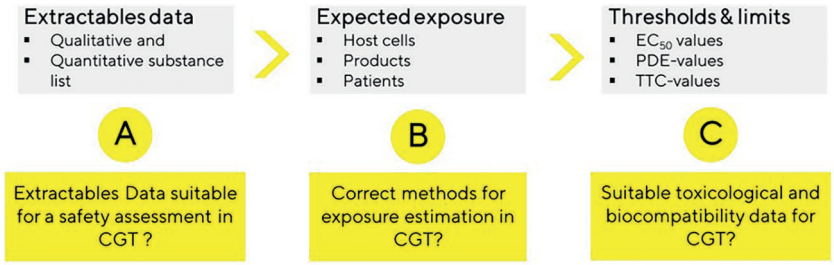

Figure 2: Workflow of an extractables assessment and the 3 challenging questions regarding an appropriate extractables assessment in CGT.

efficacy, and an increased risk to patient health. [1] To mitigate these risks, a safety assessment must consider both the therapeutic cells and the liquid in which the cells are suspended. In addition, appropriate biocompatibility tests for cells used in CGT are required to detect possible detrimental effects of PERLs on the cells manipulated *ex vivo*. [1] Available toxicity test data and biocompatibility tests are only partially applicable to cell-based therapeutics. [1,10]

In CGT applications, there is essentially no dedicated downstream purification other than a few wash steps. Notably, downstream processes for protein-based products are very efficient in removing impurities including PERLs, resulting in highly purified drug substances. [9] As a result, patient exposure to PERLs is usually very low. In contrast, because of the need to preserve cell integrity, there are few opportunities to remove impurities and PERLs from CGT products. The differences between traditional biopharmaceutical and CGT manufacturing processes raise important questions to which extent existing extractables data, exposure calculation methods, toxicological and biocompatibility data and assessment methods can be applied to CGT applications (fig. 2).

2. Extractables data

Today extractables data for SUS are frequently available in qualification documents from SU suppliers, some of them are even published in the scientific literature. [12,13,14,15,16,17] They are commonly resulting from standardized extraction studies conducted at 40 °C with defined surface to volume ratios (6/1 cm^2/mL and 1/1 cm^2/mL) and different but defined solvent systems (pure EtOH, EtOH/water 50 %, water, low and high pH). [18,19,20,21] Typical contact times are 24 h, 21 days or 70 days. Sometimes data is available for both, short and long extraction times but even some data for entire extraction kinetics or rinsing studies are reported. [22,23] Notably, today some suppliers of SUS are able to provide comprehensively elucidated extractables profiles for their SUS, can scale the data for devices of any size and combine them for assemblies. [24] Such data allow to link the qualitative extractables information with physiochemical properties and to compare the quantitative information with toxicological data as required in safety assessments.

Extractables data from standardized tests are suitable to be used in safety assessments if the extraction conditions are exaggerated over the in-use conditions.[6] CGT manufacturing requires physiological conditions, that means process temperature will not exceed 40 °C and pH ranges are close to neutral. Surface to volume ratios are commonly lower than in extractables studies. For CGT mainly aqueous serum and serum free media and buffered aqueous process fluids are used, apart from dimethyl sulfoxide (DMSO) which is used as cryo-preservative in concentrations of up to 10 %. However, since aqueous DMSO solutions are weak solvents for typical extractables permits to use extractables data from extraction with 50 % aqueous or pure EtOH as surrogates in safety assessments. Thus, one can state that extractables profiles for SUS obtained in standardized tests, are in principle appropriate to be

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Table 1

Processes relevant to establish a digital twin model for a perfusion bioreactor (fig. 3) and their mathematical description.

Process	Description	Mathematical description	Remark
1	PERLs present in the SUS plastic phase	$c_{p,0} = c^*_{l,Extr}(V^*_l/V^*_p + K^*_{p/l})$	Determined from long contact time extractables data if not known [22]
2	PERL dissolved in the liquid phase	$c_{l,diss} = \frac{c_{p,0}V_p}{K_{p/l}V_p + K_D m_{bio} + V_l}$	PERL equilibrium condition at plastic-liquid interface and at t = ∞, respectively
3	Migration of PERL from (or into) plastic phase	$\frac{\partial c_l}{\partial t} = D\left(\frac{\partial^2 c}{\partial x^2}\right)$ and $F = D\left(\frac{dc_p}{dz_p}\right)$	Ficks 1st and 2nd law describe migration of PERLs in plastic phase & trough interfaces [22,28,29]
4	Flow through the system	$\frac{dc_l}{dt} = k_Q(c_{l,in} - c_l)$	$k_Q = Q/V_l$, the perfusion rate
5	PERL adsorption on therapeutic cells	$c_{cell} = K_D \cdot c_{l,diss}$ with $c_{l,diss} = c_l \frac{1}{(1 + (m_{bio}/V_l) \cdot K_D)}$	Assuming a reversible and instantaneous adsorption of PERLs on the cells
6	Growing number and weight of biomass (therapeutic cells)	$\frac{dm_{bio}}{dt} = k \cdot m_{bio} \cdot (m_{max} - m_{bio})$	Assuming a logistic growth function (Verhulst type)

* From standardized extractables experiments

used in safety assessments of CGT applications. Additional test, such as DMSO extractions are not required. [25]

3. PERL exposure in CGT applications

The accurate estimation of PERL exposures to host cells – or therapeutic cells – to cell products and ultimately to patients is essential, in order to be compared with PERL thresholds and limits.[26] Depending on the assessment objective, these thresholds and limits can be EC_{50}-values, for the evaluation of effects on cells, permitted daily exposure (PDE) values for the evaluation of patient safety for identified compounds and threshold of toxicological concern (TTC) for unidentified compounds, respectively. Algorithms for extrapolating SUS extractables data to exposure data under static in use conditions are available and their applicability was recently shown.[22,24,27] These methods allow to scale and combine extractables data including a temperature adjustment. However, CGT manufacturing is characterized by *dynamic* conditions, making an exposure estimation challenging. The distribution of PERLs between the liquid and the plastic phase must additionally consider the temporal distribution of PERLs between the liquid and suspended cellular phase. Accurately factoring in the cell

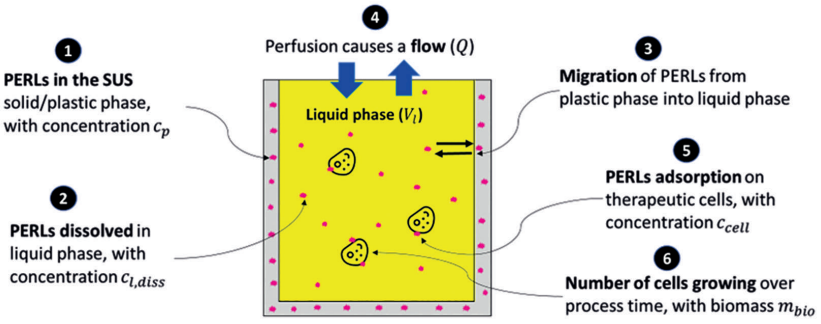

Figure 3: Scheme of a model perfusion bioreactor with 6 relevant processes for digital twin development to estimate PERL concentration.

Table 2

Overview on relevant input data required to set up a model calculating exposure for 2,4-DtBP in a perfusion bioreactor.

Required data	Source of parameter and/or data	Abbreviation	Values for example
Surface of SUS (contacting process liquid)	Technical drawing	S_p	1,200 cm^2
Volume or weight of SUS	Technical drawing or weighing	V_p	48 cm^3
Thickness of SUS, max. diffusion length (perpendicular to interface)	Technical drawing	z_p	0.04 cm
Volume of process liquid	Process parameter	V_l	1,400 cm^3
Cultivation time	Process parameter	t	22 days
Perfusion rate	Process parameter	k_Q	0.075/h
Initial 2,4-D*t*BP concentration in the plastic of the SUS	Extractables studies or empirical data [22]	$c_{p,0}$	104 µg/cm^3
2,4-D*t*BP conc. in liquid phase, t = 0	Empirical process related data	$c_{l,0}$	0.2 µg/mL
2,4-D*t*BP conc. in inflow	Empirical process related data	$c_{l,in}$	0.2 µg/mL
Diffusion coefficient of 2,4-D*t*BP in plastic phase	Literature, experimental data, or estimation methods [22,29,31]	D_p	$3 \cdot 10^{-10}$ cm^2/sec
2,4-D*t*BP partition coefficient between plastic and liquid phase	Derived from extractables data, or via estimation methods [22,32,33]	$K_{p/l}$	13
2,4-D*t*BP partition coefficient between liquid phase and biomass	Estimated based on experimental data [30,34]	K_D	0.02 L/g
Biomass ratio for t = 0 → 20 days	Growth functions or empirical process related data	m_{bio}/V_l	1–50 g/L

growth, media and/or reagent additions, washing steps and perfusion flow rates adds complexity to exposure estimation required in CGT. Furthermore, a direct measurement of PERLs as trace contaminants adsorbed on cells or dissolved in the liquid phase is extremely difficult if not impossible.

An alternative to analytical testing is to model the interaction of PERL with cells *in silico* using so called *"digital twins"*. Digital twins are IT systems allowing to virtually simulate physical, chemical, and biological processes in SUS. They are a reduction of real world-systems to those elements and processes which are relevant for an anticipated evaluation. A digital twin of a perfusion bioreactor is schematically presented in fig. 3. The anticipated output of such a virtual perfusion bioreactor is the temporal development of the PERL concentration in the liquid phase, $dc_{l,diss}/dt$ and adsorbed on the suspended cellular phase, i.e., the biomass, dc_{cell}/dt. And with that, giving access to relevant exposure data for patients and the *ex vivo* manipulated cells over the entire process time. The development of a digital twin of a bioreactor can be divided into 3 steps, which are briefly described in the following.

In a first step, processes responsible for the temporal development of the PERL concentration in the different phases of the bioreactor (i.e., $dc_{l,diss}/dt$ and dc_{cell}/dt) are identified. For a perfusion bioreactor at least 6 relevant processes can be identified and are schematically presented in fig. 3.

In a next step, these processes need to be expressed as mathematical equations, which describe their temporal development, their inter-relation and eventually the equilibrium conditions of the system (table 1). For establishing the virtual perfusion bioreactor, a system with complete cell retention is considered here.

In a third step the mathematical descriptions of the processes are combined in a differential equation, which allows to maintain the mass balance. It can be generically written as:

$$\frac{dc_{l,diss}}{dt} = \left(\sum_{n=1}^{i} sources - \sum_{p=1}^{j} sinks\right)/V \qquad \text{Eq. 1}$$

In case of a perfusion bioreactor, sources for PERLs are the amount of PERLs entering the system with the inflow and the PERLs released from the different components of the SUS. Sinks for PERLs are the potential migration of PERLs into SU components, the absorption of PERLs on the biomass and the terminal sink of PERLs with the outflow. Further sinks can be degradation reactions or, in case of a bioreactor also metabolization of PERLs, but these processes are not considered in the example presented here. To establish the digital twin, the above equations (table 1) are

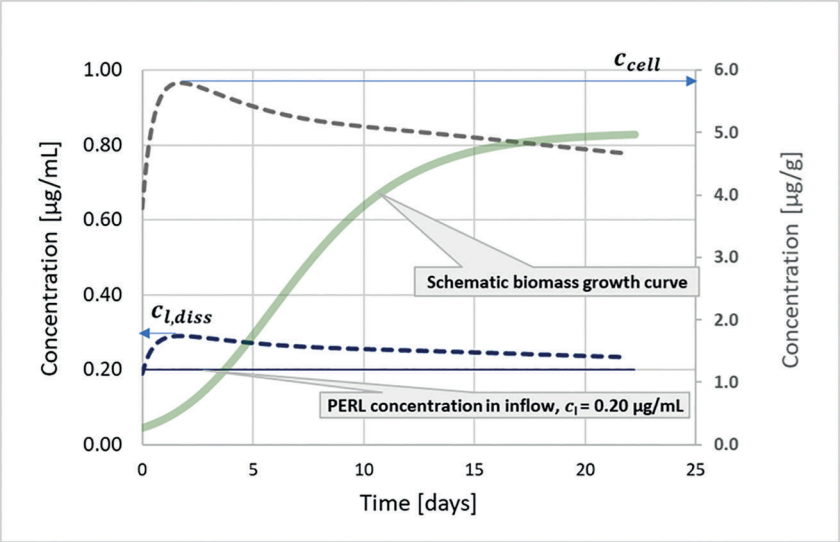

Figure 4: Modeling results of 2,4-DtBP exposure in a CGT perfusion bioreactor to the liquid phase (blue dashed curve) and the cellular phase (grey dashed curve). The green sigmoid curve represents a hypothetic cell growth used in this model. The flow is much higher than release rates of PERLs indicating a strong wash out effect; an accumulation of PERLS in the liquid and/or cellular phase does not occur.

– in a last step – translated into a computer program, which allows to numerically solve Eq. 1.

In table 2 the required model input data and their sources are summarized. It includes data like the SUS dimension and geometry, but also process information, such as run time, perfusion flow rate and temperature. These data can be easily and accurately obtained, as they are given in technical drawings of the SUS or are qualified process parameters. The initial PERL concentration in the polymer is either known, can be determined with an in-polymer analysis, or is calculated from extractables data. Diffusion and partition coefficients for the PERL can be found in the literature, calculated with estimation methods, or be derived from experimental data, e.g., kinetic extractables studies. For the cellular phase, in table 1, a logistic growth function is suggested, in this case the parameters for this function need to be included. It should be noted that other growth functions or even empirical data – including such from on-line measurements – can be used in such models. Irrespectively of the used growth function, an adsorption coefficient for PERL on the cells is required. This parameter can so far only be obtained from dedicated experiments. [30]

In fig. 4 modelling results for the extractables 2,4-Di-*tert*-butylphenol (2,4-D*t*BP) in a virtual perfusion bioreactor are shown. The respective model utilizes the parameters given in table 2 and predicts the concentration development of 2,4-D*t*BP in the liquid phase and adsorbed on the cells over 22 days.

With this model, it was possible to closely replicate the CGT production environment and forecast how the concentration of a potential PERL varies over time in both, the liquid phase and adsorbed to the cells. The PERL concentration, which would be conventionally used as exposure value in an E&L safety assessment, considers only the equilibrium concentration of the PERL in the bioreactor without biomass and without flow (calculated from extractables data with Eq. 1 and 2 in table 1 and data from table 2) (see Eq. 2 below).

By additionally considering the presence of the biomass – still without perfusion – the maximum exposure concentration under equilibrium conditions for liquid and cellular phase can be calculated with the Eq. 2 and 5 in table 1 and data from table 2, respectively (see Eq. 3 and Eq. 4 below).

In opposite to this conventual exposure estimation, the model calculation in fig. 4 returns a significantly lower 2,4-D*t*BP concentration in the liquid phase during the entire cultivation time of 20 days, far from approaching the static equilibrium concentration. Moreover, the curve for the liquid phase in fig. 4 shows a maximum concentration after 1½ days of cultivation with approx. 0.3 µg/mL followed by a declining 2,4-D*t*BP concentration with time. With that, the model calculation indicates that the PERL in the liquid phase is dominated by a dilution or "wash-out" effect caused by the perfusion. Extrapolating the curve in fig 4 to longer cultivation times, one can anticipate a concentration of 2,4-D*t*BP converging eventually to the inflow concentration of 0.2 µg/mL. The 2,4-D*t*BP concentration adsorbed on the cells does not reach the static equilibrium concentration and is less than 5 µg/g after 20 days of cultivation. The 2,4-D*t*BP concen-

$$c_{l,diss} = \frac{c_{p,0}V_p}{K_{p/l}V_p+V_l} = \frac{104\ \mu g/cm^3 \cdot 48\ cm^3}{13 \cdot 48\ cm^3+1{,}400\ cm^3} = 2.47\ \mu g/cm^3\ \textit{or}\ 2.47\ \mu g/mL \qquad \text{Eq. 2}$$

$$c_{l,diss} = \frac{c_{p,0}V_p}{K_D V_p+K_D m_{bio}+V_l} = \frac{104\ \mu g/cm^3 \cdot 48\ cm^3}{13 \cdot 48\ cm^3+0.02 L/g \cdot 20\ g/L \cdot 1{,}400\ cm^3+1{,}400\ cm^3} = 1.93\ \mu g/cm^3\ \textit{or}\ 1.93\ \mu g/mL$$
Eq. 3

$$c_{cell} = K_D \cdot c_{l,diss} = 0.02\ L/g \cdot 1.93\ \mu g/mL = 39\ \mu g/g. \qquad \text{Eq. 4}$$

tration on the cells is influenced by the wash out effect as well. Simulating different cultivation scenarios lead consistently to similar results: The media flow rate through the system is much higher than the release rates of PERL resulting in a strong wash out effect and therefore no accumulation of PERL in the liquid phase nor on the cellular phase is expected in perfused systems. Interestingly, the curves for dissolved and adsorbed 2,4-D*t*BP are not synchronized but different in their development. This is due to the growing biomass, modeled here with a sigmoidal growth function.

Calculations like the above demonstrates: (1) Conventional safety assessments using equilibrium data and ignoring the cellular phase is operating with unrealistic high PERL exposure values in the liquid phase, (2) including the cellular phase in equilibrium calculations, overestimates the potential exposure for the liquid and the cellular phase and (3) that even with simple models a plausible and more realistic PERL estimation in both phases is possible. Thus, model calculations can be usefully applied to determine the PERL exposure in cases where an analysis is not feasible, for example in harvest steps, or – even more relevant in future – to estimate the PERL exposure for therapeutic cells and simultaneously the patient exposure with the therapeutic cells in CGT. [10]

4. Investigating effects of PERL in CGT using a high-throughput cell painting assay

Concerns are associated with PERLs from SUS eventually reaching a final product and negatively impact product quality and/or patient safety. In this context patient safety assessment are commonly carried out following principles analogous to toxicological assessments of drug products (DP) impurities. Exposure values are calculated down to the daily dose of the DP and compared with permitted daily exposure (PDE) values for identified PERL and threshold of toxicological concern (TTC) for unidentified PERLs to allow for the evaluation of patient safety (fig. 2). [26]

In the area of cell and gene therapy (CGT) this approach is not sufficient and tools are required to provide a complete picture including also potential effects of PERLs on the therapeutic cells manipulated *ex vivo*. [1] PERLs can interact with cells and have the potential to affect many aspects of cell health, metabolism and function of cells. [30,35,36] Yet, metrics such as PDE or TTC are unable to evaluate effects on isolated human cells. PDE and TTC are derived from toxicity tests performed on mammalian test animals (e.g., rats or mouses), and non-human cell lines. Consequently, inter-species transferability and the transfer of systemic toxicological endpoint to endpoint on a cellular level as required in CGT is difficult if not impossible. Available tests for biocompatibility of SUS that assess only one or a few end points are not sufficient for CGT applications. [37,38,39,40,41] They can easily miss subtle changes in cell phenotype and are biased towards specific endpoints.

Moreover, for SUS used in CGT, demonstration of the absence of any effect to the cells is a reasonable technical requirement. Researchers and process engineers implicitly expect, that the process equipment they are using is generally *inert* and does not influence their experimental work and desired processes in any unforeseeable way. [42]

To address these shortcomings, the authors partnered with the Max Planck Institute (MPI) in Dortmund to assess PERLs' impact on living cells using a high throughput cell painting assay (HT-CPA, fig. 5). [10] HT-CPA is an unbiased phenotypic profiling technique that multiplexes six fluorescent dyes to analyze as many as 1,700 features on a cell-by-cell basis using automated high-content cell imaging and analysis with an approved profiling methodology (*https://cellprofiler.org/*). [43,44] The MPI database can link 579 cell features out of the list of 1,700 features with a known effect, associated with a chemical entity. [45] The HT-CP assay provided a powerful and sensitive means to detect stimulating and detrimental effects using only a small number of cells and a low amount of extract.

45 known extractables were selected from the ExSim database and subjected to the HT-CPA (table 3). [24] The tested substances range from plastic antioxidants and their typical degradation product to an antistatic agent, plasticizers, photoinitiators and polymer monomers and oligomers.

The test substances were chosen based on the following criteria: (1) The substance was unambiguously identified in extractables tests on SUS conducted in the last years; and (2) the substance should have a sufficient water solubility, to allow application in a biological test system. The compounds were applied to the cells in concentrations of 10–50 µM which corresponds to 0.7–23.7 µg/mL, respectively. Additionally, we took two SUS extracts obtained from standard extractables tests, namely a sterile filter and a tube, extracted with pure EtOH and EtOH 50 % over 24 h and 21 days at 40 °C respectively. The results of the HT-CPA can be summarized as follows:

- 41 out of the 45 selected extractables and both SUS extracts (table 3) did not show any effect on any of the 579 cell features above the defined threshold.
- Only 4 out of the 45 selected extractables had a measurable effect on any of the cell features above the defined threshold (table 4). The observed weak effects on histone deacetylase, DNA synthesis, and cholesterol homeostasis, were consistent with previously reported negative

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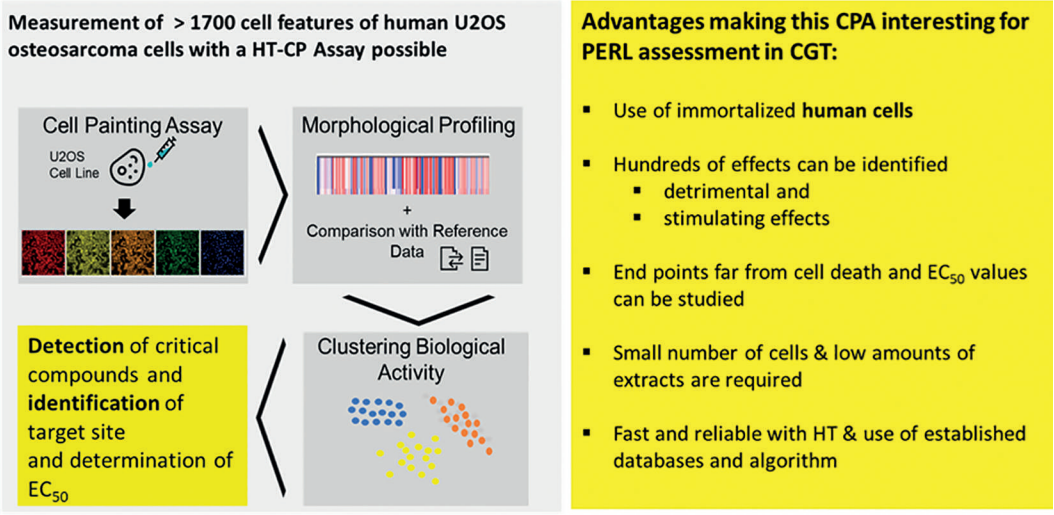

Figure 5: Workflow of the high throughput cell painting assay for studying effects of PERLs on human cells.

Table 3

Overview on the extractables substances and the two SUS extracts tested with the HT-CPA.

Compound	CAS
12-Aminododecanolactam (Laurinlactam)	947-04-6
1,3:2,4-Bis(3,4-dimethylobenzyl(ideno)sorbitol (Millad 3998)	135861-56-2
1,3-Di-*tert*-butylbenzene, 99%	1014-60-4
1,4-Cyclohexane dimethanol	105-08-8
1-Acetyl-2-pyrrolidone	932-17-2
1-Dodecanol	112-53-8
2 Mercaptobenzo thiazole	149-30-4
2 Pentanone; 99%	107-87-9
2,4-Di-*tert*-butylphenol, 99%	96-76-4
2,6-di-*tert*-butyl-1,4-benzoquinone	719-22-2
2-Pyrrolidone	616-45-5
3-(3,5-Di-*tert*-butyl-4-hydroxyphenyl) propionic acid (Fenozan)	20170-32-5
3,3'-Dinitrobisphenol A	5329-21-5
3,4-Dimethylbenzaldehyd 98%	5973-71-7
3,5-Di-*tert*-butyl-4-hydroxybenzaldehyde	1620-98-0
4-Hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine	52722-86-8
4-n-Nonylphenol	104-40-5
7,9-Di-*tert*-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	82304-66-3
Acetophenone	98-86-2
Aniline	62-53-3
Benzyl alcohol 99,8%	100-51-6
Bis(2-hydroxyethyl)terephthalate (BHET)	959-26-2
Bis(2,4-di-*tert*-butylphenyl)phosphate (bDtBPP)	69284-93-1
Bis-(4-chlorphenyl)-sulfon	80-07-9

Compound	CAS
Bisphenol A (BPA)	80-05-7
Butylhydroxytoluene (BHT) (2,6-Di-*tert*-butyl-4-methylphenol)	128-37-0
Caprolactam	105-60-2
Dibutylphthalate	84-74-2
Diphenyl carbonate	102-09-0
Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO)	75980-60-8
Ethyl 4-ethoxybenzoate	23676-09-7
Glycerol	56-81-5
Hexanal	66-25-1
Methyl ethyl ketone (MEK)	78-93-3
N-Butylbenzenesulfonamide	3622-84-2
N-Lauryldiethanolamine	1541-67-9
N-Methyl-2-pyrrolidone	872-50-4
Octamethyl cyclotetrasiloxane (D4)	556-67-2
Octanoic acid	124-07-2
Phenol	108-95-2
p-Tolualdehyde 98% (4-Methyl benzaldehyde)	104-87-0
p-Toluenesulfonamide	70-55-3
tert-Butanol (*tert*-Butyl alcohol)	75-65-0
PVP-Copolymer	25086-89-9
PVP after gamma irradiation	25086-89-9_g
Ethanol extract of PESU filter cartridge	(SUS Extract)
50% Ethanol extract of silicone tubing	(SUS Extract)

biocompatibility effects of these compounds. [36,46]

- Only one compound, the antistatic agent *N*-Lauryl-diethanolamine showed a significant detrimental effect on cell growth (table 4), which was unreported so far.

5. Conclusion

Our investigation into extractables data, digital twins, and HT-CPA technologies shows promising results. PERL exposures can be plausibly modeled using existing extractables information, and the impact of PERL on human cells can be evaluated in a fast and cost-effective way. The dynamic modeling approach calculates significantly lower PERL exposure values than static equilibrium-based calculations or worst-case assumptions. The findings also provide reassurance that SUS are well-suited for CGT applications and potentially detrimental compounds can be confidently identified. This allows SUS suppliers to avoid them for SUS manufacturing and consequently eliminate them as potential leachables in CGT bioprocesses. In the long term, the information gained can be used to inform the manufacturers of SUS to further improve compatibility with CGT applications. HT-CPA was identified as a powerful tool for screening and investigation of effects of PERLs on human cells. It could be shown that 41 out of 45 common extractables did not show

Table 4

Overview over the four substances, which showed an effect in HT-CPA.

Substance	Function	Induction in CPA	Cluster Similarity
bD*t*BPP	Degradant antioxidant	Low	DNA synthesis
N-Lauryldiethanolamine	Antistatic agent	High	Cholesterol homeostasis
4-*n* Nonylphenol	Plasticizer	Low	DNA synthesis
TPO	Photo-initiator	Low	Histone deacetylase

any effect in the HT-CPA. On the other hand, HT-CPA identified 4 compounds – out of the 45 – which were partially known as potentially detrimental to cells. Together, these results demonstrate the sensitivity of the HT-CP assay to detect potential "trouble-makers" and support the conclusion that SUS are in general sufficiently biologically inert and therefore well-suited for use in CGT applications.

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