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- [1] M. Hammond, H. Nunn, G. Rogers, H. Lee, A.-L. Marghitoiu, L. Perez, et al., Identification of a leachable compound detrimental to cell growth in single-use bioprocess containers., *PDA J. Pharm. Sci. Technol.* 67 (2013) 123–34. doi:10.5731/pdajpst.2013.00905.
- [2] J. Kadarusman, R. Bhatia, J. McLaughlin, W.R. Lin, Growing cholesterol-dependent NSO myeloma cell line in the wave bioreactor system: Overcoming cholesterol-polymer interaction by using pretreated polymer or inert fluorinated ethylene propylene, *Biotechnol. Prog.* 21 (2005) 1341–1346. doi:10.1021/bp050091+.
- [3] R. Eibl, N. Steiger, C. Fritz, D. Eisenkrätzer, J. Bär, D. Müller, et al., Recommendation for Leachables Studies - Standardized cell culture test for the early identification of critical films, 2014.

An approach to evaluate new single-use film types

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Introduction

Single-use bioreactors as well as vessels for storage, sampling, freezing or mixing which are made of flexible bags have been well-established in biopharmaceutical production. Their multiple advantages are well-known, whereas the most frequently mentioned one is the low contamination rate of only around 1%. However, some developers and producers of biopharmaceuticals refuse their usage because of potential interactions of the plastic material with cells or proteins. Apart from extractables, leachables are particularly relevant, as they can migrate from the film contact layer into the process fluid under process conditions and can inhibit cell growth or impair product quality. Leachables (such as bDtBPP [1]) derive from additives and are formed during the bag manufacturing process or gamma sterilization. A further reason for product quality inhibition can be the adsorption of hydrophobic medium components (like fatty acids or cholesterol [2]) to the contact layer of the polyethylene film.

Consequently, there is a high demand for the early identification of critical film types. A new standardized cell-based assay has been recently published by DECHEMA [3], which allows general recommendations and a comparison of different film materials by means of cell growth studies with sensitive CHO cell lines and a chemically defined minimal culture medium.

Evaluation of new single-use bag materials

For the evaluation of a new film type, the test procedure recommended by the DECHEMA was extended. Growth performance studies were carried out with five different cell lines (Fig. 1) from animal or human origin, which are relevant for biopharmaceutical production. The corresponding media, reaching from chemically defined minimal media to serum-reduced media, were incubated with a test film, a negative control film and a reference (DURAN glass bottle) under defined conditions (Fig. 1). Subsequently, the media were used for growth experiments in shake flasks, spinner flasks and TubeSpin bioreactors. The procedures are described in detail in [4].

Compared to the test film, the negative control has a 30-fold higher concentration of TBPP, which degrades to the toxic leachable bDtBPP. The films were provided as gamma-irradiated test bags. A surface-to-volume ratio of 3 cm²/mL was applied for the extraction.

Additionally, the adsorption of hydrophobic medium components to the film contact layer was examined. This was realized by using a cholesterol-dependent NS0 cell line. The leachables extraction was carried out (a) with the standard TurboDoma medium and (b) with medium without the TurboDoma-related lipid components. Before inoculation, lipid components were added to the lipid-free medium extract (Fig. 1).

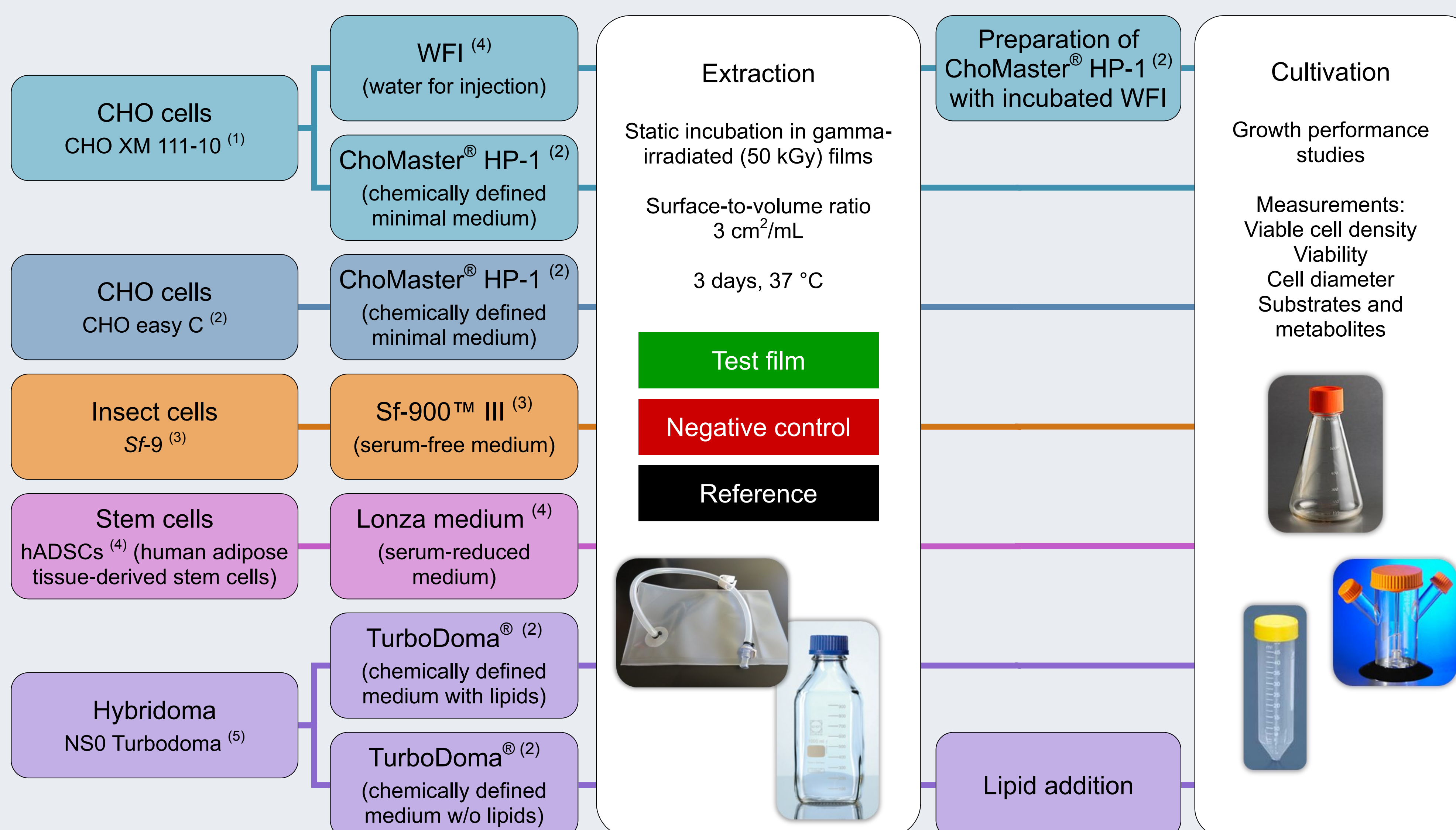


Fig. 1: Process flow chart of the leachables study test procedure.

(1) Culture Collection of Switzerland AG, Switzerland, (2) Cell Culture Technologies LLC, Switzerland, (3) Gibco Invitrogen, USA, (4) Lonza Ltd., Germany and USA, (5) Medical Research Council, United Kingdom.

Conclusions

- Nearly identical growth behavior of cells between test film and reference irrespectively of the cell line (Fig. 2)
- Significantly lower viable peak cell densities with the negative control film indicating the presence of leachable substances
- Sensitivity to growth-inhibiting substances varies among the cell lines and media
 - High sensitivity for the CHO cell lines and the NS0 cells in chemically defined (minimal) media: strong decrease of viability, 65-69% lower peak cell concentration in the negative control compared to the reference, see Fig. 2 and Fig. 3
 - Less sensitivity was for the Sf-9 cell line grown in serum-free medium: only 15% lower peak cell density, no decrease in viability
- Lipid adsorption to the contact layer of the test film could be excluded for the studies with hybridoma cells
- Further investigations required to conclude whether lipid adsorption or leachables are responsible for growth inhibition in the negative control film (Fig. 2 E)

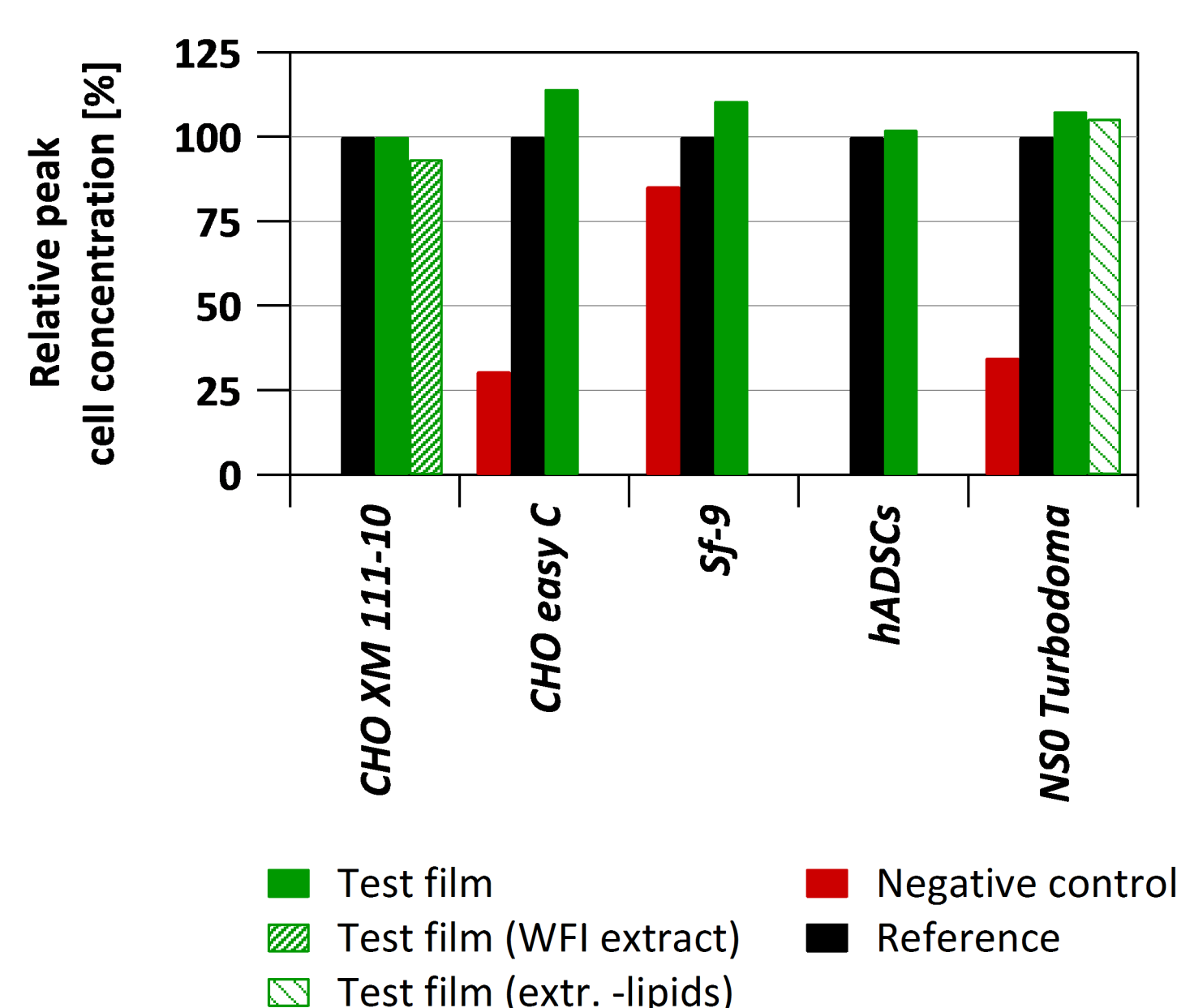


Fig. 3: Achieved peak cell concentrations in the growth experiments relative to the reference.

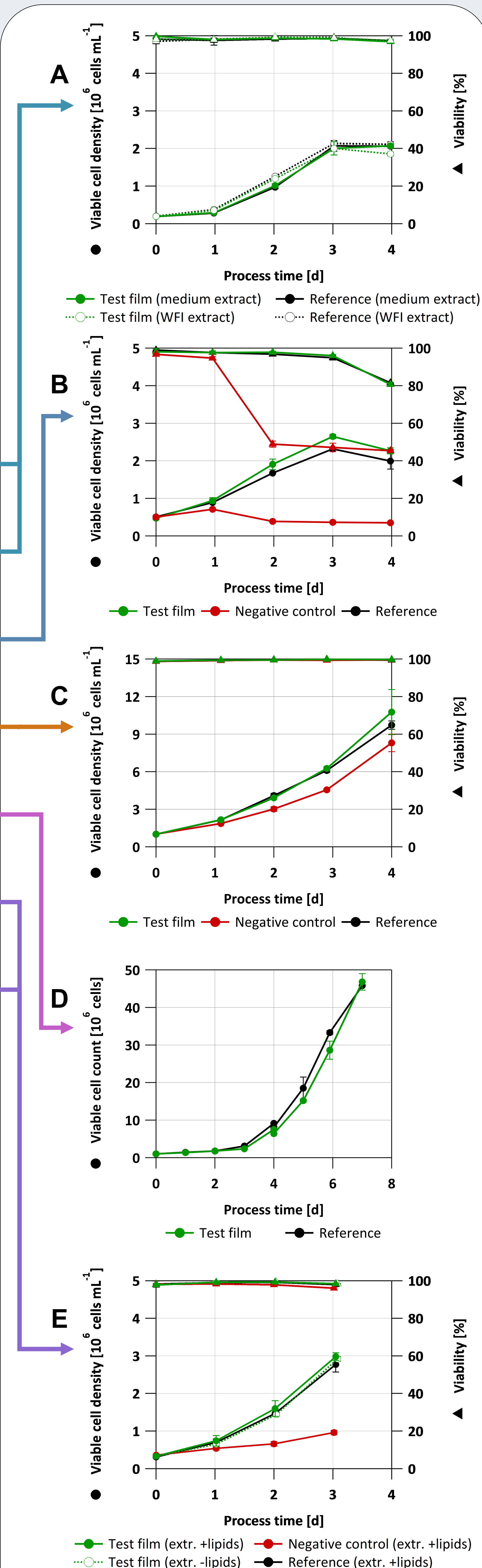


Fig. 2: Results of the growth performance studies with different cell lines. (A) CHO XM 111-10, (B) CHO easy C, (C) Sf-9, (D) hADSCs, (E) NS0 Turbodoma.

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