Virus production in single-use bioreactor systems using pre-sterilized microcarriers

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Virus production in single-use bioreactor systems using pre-sterilized microcarriers

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Abstract

In this study, we describe different approaches for the cultivation of Vero cells, grown to high cell densities using Cytodex™ and/or Cytodex Gamma microcarriers in serum-free cultivation medium, using ReadyToProcess WAVE™ 25 and/or Xcellerex™ XDR-10 single-use bioreactor system.

It is known that depending on the bioreactor design, operating parameters need to be carefully chosen to avoid excessive shear, which can otherwise be deleterious to cell growth and virus production [1, 2].

By using ready-to-use Cytodex Gamma microcarriers, the time-consuming step of microcarrier preparation and sterilization prior to culture was removed. Instead, the microcarriers could be added directly to the bioreactor. Three different culture conditions were used to compare Cytodex and Cytodex Gamma microcarriers. Virus yields were determined by 50% tissue culture infective dose measurement ($TCID_{50}$). A viral titer of 10^7/mL was observed for all three cultures.

These results offer valuable information to facilitate design-in of single-use bioreactor systems and ready-to-use Cytodex Gamma microcarriers, enabling flexible future vaccine productions at smaller scales.

Introduction

Since the 80´s, microcarriers have been used to provide growth support for adherent cells in vaccine biomanufacturing processes at scales up to 6000 L. Today, as yields are increasing, most newly developed vaccines are manufactured at smaller scales, making single-use technologies suitable. Because many anchorage-dependent cell lines grown on microcarriers are sensitive to shear stress, some of the remaining challenges are the need for homogenous well-suspended microcarriers and efficient oxygenation of the medium. These prerequisites can limit operating conditions and volumes of the bioreactor process. By selecting operating parameters carefully, good cell growth and virus yields can be achieved in a single-use bioreactor system.

Materials and methods

XDR-10 bioreactor system (GE Healthcare)

10 L XDR Cellbag™ prototype for microcarriers (GE Healthcare)

ReadyToProcess WAVE 25 bioreactor system (GE Healthcare)

10 L Cellbag bioreactors (GE Healthcare)

Spinner flask 125 mL (Techne)

Cytodex microcarriers (3 g/L) (GE Healthcare)

Cytodex Gamma microcarriers (3 g/L) (GE Healthcare)

HyClone™ SFM4MegaVir and OptiPRO™ SFM cell culture media (GE Healthcare and Thermo Fisher Scientific, respectively)

Vero cells (ECACC)

Influenza virus (Influenza A/Solomon Islands/3/2006/ H1N1) (ATCC)

Virus yields were determined by $TCID_{50}$ measurement.

To support cell culture for production of vaccines and minimize bead handling procedures (hydration, sterilization, transfer to reactor), a packaging and connectivity solution of a sterile, ready-to-use, gamma irradiated version of Cytodex microcarriers was developed (Fig 1) [3].

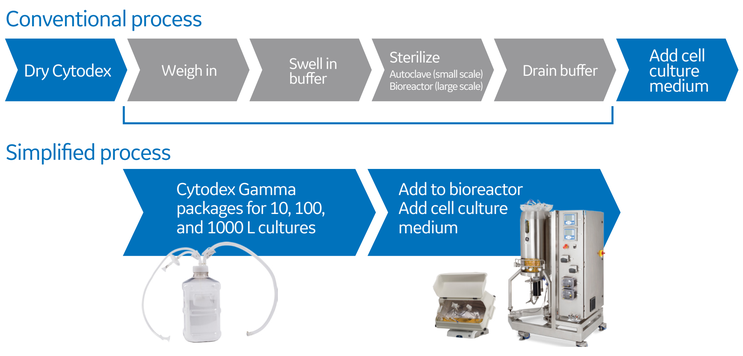

Fig 1. Illustration of a simplified process using sterile, ready-to-use Cytodex Gamma microcarriers, which are packaged in a single-use container to allow easy transfer to various single-use bioreactor systems.

Results

Comparable results in cell growth and cell concentrations were observed when using microcarriers (Cytodex or Cytodex Gamma) in a single-use bioreactor system (ReadyToProcess WAVE 25 [4] or Xcellerex XDR-10 single-use bioreactor system) (Fig 2 and 3).

We show that by carefully selecting operating parameters, good cell growth and virus yields can be achieved in a single-use bioreactor system. Furthermore, the time consuming process of preparation and sterilization of the microcarriers prior to culture can be simplified significantly by using Cytodex Gamma microcarriers.

Influenza virus propagation in Vero cells

Vero cells grown on microcarriers (Cytodex or Cytodex Gamma) were infected with influenza virus during exponential growth phase at approximately 1×10^6 cells/mL [5]. The concentration of infectious virus was determined by $TCID_{50}$ (Table 1). Figure 4 shows cell morphology at time of infection (TOI) and harvest (TOH).

Table 1. $TCID_{50}$ result for single-use bioreactor cultivation

Microcarrier	$TCID_{50}$	Bioreactor system
Cytodex 1	$10^{7.0}$/mL	ReadyToProcess WAVE 25 (2 L)
Cytodex 1 Gamma	$10^{7.3}$/mL	ReadyToProcess WAVE 25 (2 L)
Cytodex 3	$10^{7.1}$/mL	ReadyToProcess WAVE 25 (4 L)
Cytodex 3 Gamma	$10^{7.5}$/mL	ReadyToProcess WAVE 25 (4 L)
Cytodex 3	$10^{7.6}$/mL	XDR-10 (6 L)
Cytodex 3 Gamma	$10^{7.4}$/mL	XDR-10 (6 L)

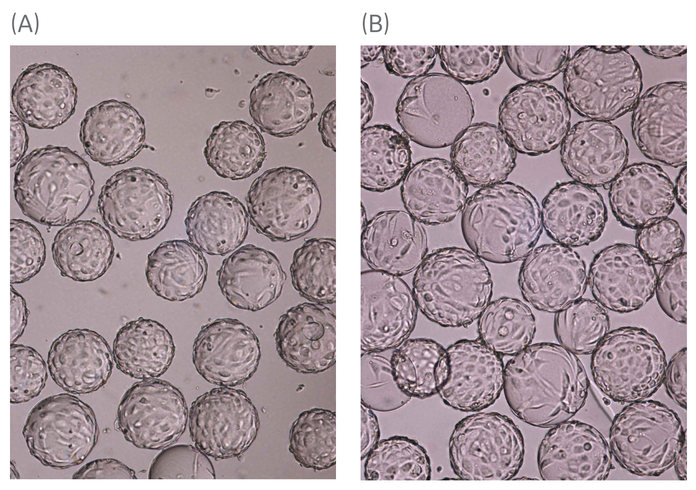

Fig 2. Vero cells perform equally well both on (A) Cytodex 1 Gamma and (B) Cytodex 3 Gamma regarding distribution and cell growth after 72 h.

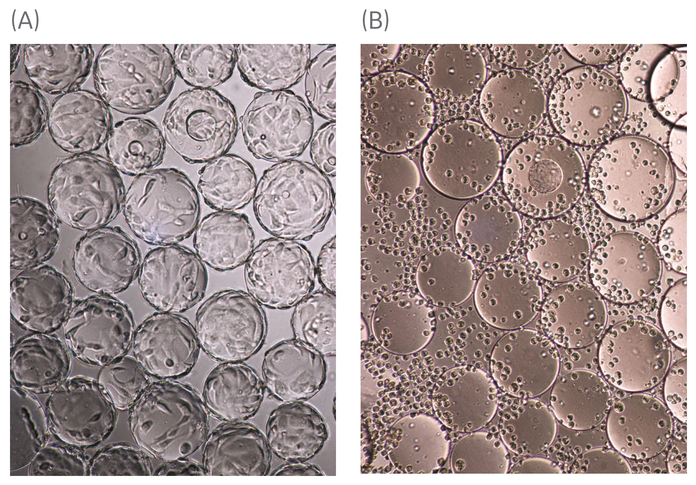

Fig 4. Morphology of Vero cells on Cytodex 1 Gamma (A) when reaching cell confluence (TOI); (B) after 72 h infection (TOH) when cytopathic effect was shown.

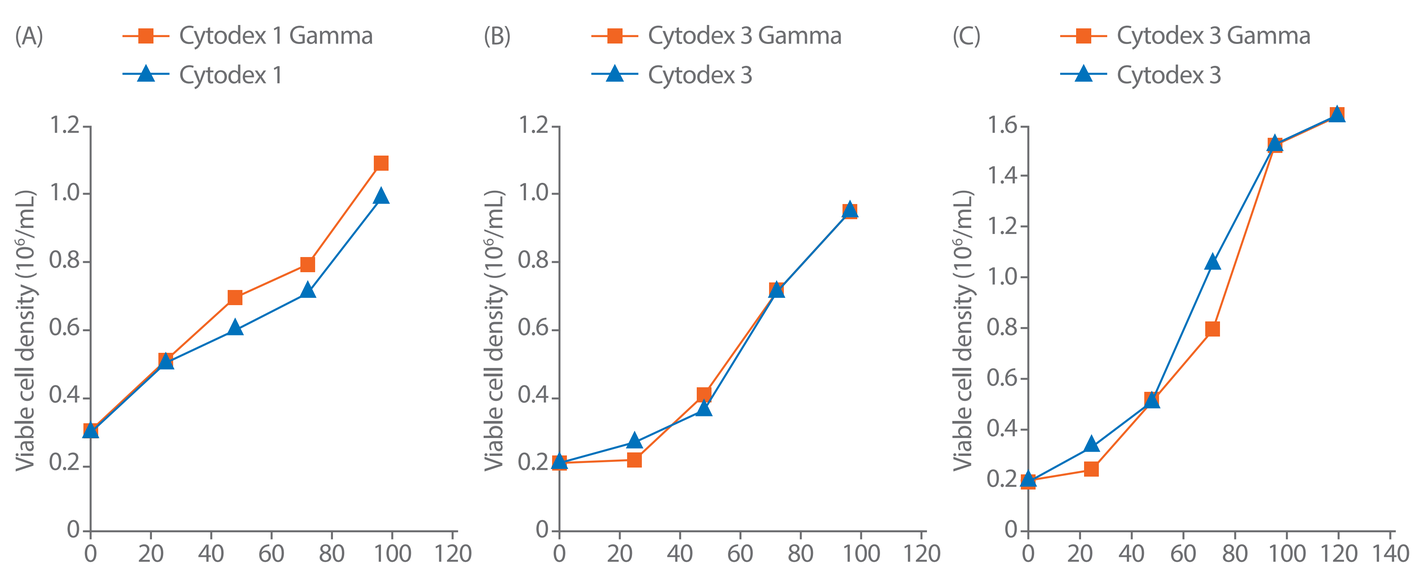

Fig 3. Comparison between (A) Cytodex 1 and Cytodex 1 Gamma in ReadyToProcess WAVE 25 (2 L); (B) Cytodex 3 and Cytodex 3 Gamma in ReadyToProcess WAVE 25 (4 L); (C) Cytodex 3 and Cytodex 3 Gamma in XDR-10 (6 L). There was no observed difference in cell growth irrespective of the choice of microcarriers, volume, or single-use bioreactor system.

Conclusions

- No difference in distribution and cell growth was observed between Cytodex and Cytodex Gamma when using single-use bioreactor systems.
- The virus titer activity ($TCID_{50}$) for influenza virus was comparable for Cytodex and Cytodex Gamma.
- Both Xcellerex XDR-10 and ReadyToProcess WAVE 25 allowed simplified medium exchange, resulting in minimal loss of microcarriers.
- Cytodex Gamma Container system offered simplicity and safety based on a sterile workflow.

References

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