Application note

FlexiGrow™ Carroucell

CARROUCELL
Tailoring microcarriers

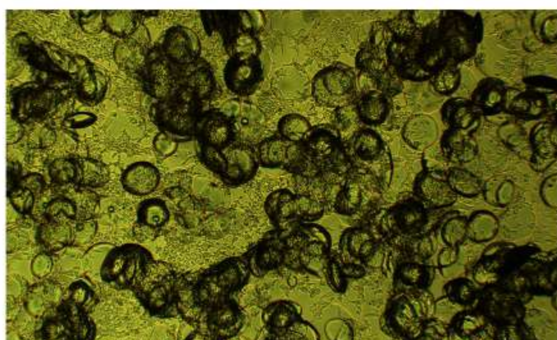
Culture of adherent cancer cells on FlexiGrow™ Carroucell microcarriers for the design of animal models in oncology.

Key words: Cell culture, Scale-up, Cancer cells, Animal model, Oncology, Culture yields, Cell quality, Resource optimization Static culture, 2D culture, FlexiGrow microcarriers, Carroucell microcarriers.

Abstract

Carroucell FlexiGrow™ microcarriers stand out as unparalleled microcarriers in the market, offering a distinctive capability to elevate yields in adherent cell cultures within static 2D environments. This study unveils an avant-garde 2-step culture protocol utilizing FlexiGrow™ microcarriers for the extensive amplification of various human adherent tumor cells. Through meticulous adjustments in FlexiGrow™ microcarrier size, concentration, and culture duration, we achieved a remarkable upsurge in the culture yields of SKOV3, Hs746t, and MDA-MB-175 cells, all while maintaining optimal cell viability.

This pioneering approach transcends conventional practices, resulting in a noteworthy reduction in space requirements, consumables, and time resources dedicated to the amplification of these cell lines. The ramifications extend to a substantial decrease in costs associated with large-scale cell production in oncology, all achieved without necessitating any significant modification to the existing production chain. Carroucell FlexiGrow™ microcarriers redefine the landscape of adherent cell culture, offering a cost-effective, efficient, and innovative solution for oncology research and production.



Introduction

The use of tumor cells as in vivo tumor models is a commonly used approach in biomedical research to study tumor development, progression, and response to treatment. Tumor models recreate a three-dimensional environment closer to reality than cells growing in 2D in vitro. They enable to better reproduce the conditions present in patients' tumors [1][2] and to better understand the mechanisms of tumor formation and progression. These models are based on tumor cell lines grown in large quantities [1] and injected into animal models such as immunocompromised mice [3]. Tumor models play a crucial role in the development of new treatments, enabling their efficacy, pharmacokinetic parameters, and potential side effects to be studied before they are tested in clinical trials [4]. As such, they are essential tools in the development of new anti-cancer treatments. To create an in vivo tumor model, many tumor cells are needed. The number of cells required for each experiment can run into the hundreds of millions.

Carroucell is a French society specialized in the synthesis of flat disc microcarriers. In comparison with spherical microcarriers of the market, Carroucell microcarriers improve cell adherence, reduce cell mortality, and increase cell harvesting at the end of the culture [5]. Recently, Carroucell developed an innovative way to use their microcarriers, no longer in agitated systems, but inside static culture environments. By including flat disc FlexigrowTM microcarriers inside those systems, Carroucell multiply the adherence surface per volume unit (Figure 1). This proprietary technology, protected by a recently granted patent, not only represents a paradigm shift but also significantly amplifies cell culture yields, extending from traditional multiwell plates to advanced multitray systems.

Antineo is a CRO providing services in preclinical therapeutic research in oncology. Antineo offer services to customers wishing to evaluate the antitumor activity of their compounds in the most relevant models, either as single agents or in combination with standard therapies, as well as in models resistant to conventional treatments. The services provided by Antineo include personalized advice and dedicated expertise to determine the most relevant models and to optimize the preclinical strategy for the future clinical development of their clients. Those services are provided by a team with international level expertise in onco-pharmacology.

A collaborative effort between Antineo and Carroucell has harnessed the potential of Carroucell cutting-edge technology for the large-scale amplification of diverse tumor cell lines. The overarching goal is to address and alleviate the spatial constraints and economic burdens associated with the preparation of animal tumor models, particularly for Antineo.

Figure 1: **Growth of adherent cells on FlexiGrow™ microcarriers.**

After their inclusion in static culture devices, FlexiGrow™ microcarriers bring additional adherence surface to the cells. This permits cells to adhere and grow both at the surface of the culture device and on the microcarriers. Along the culture, cells optimize their growth on FlexiGrow™ microcarriers and form tridimensional structures.

Materials and methods

Cells and culture medium

SKOV3, Hs746t and MDA-MB-175 cells were transferred from Antineo to Carroucell. SKOV3 cells were cultured in RPMI medium (Thermo Fisher Scientific, Illkirch, France) supplemented with 10% fetal calf serum. Hs746t cells were cultured in DMEM medium (Thermo Fisher Scientific, Illkirch, France) supplemented with 10% fetal calf serum. MDA-MB-175 were cultured in DMEM-F12 medium (Thermo Fisher Scientific, Illkirch, France) supplemented with 20% fetal calf serum. All media were supplemented with 100 units/mL penicillin and 100 µg/mL streptomycin, respectively.

Microcarriers

Carroucell FlexiGrow™ disc microcarriers (batch 01-09-2023) with respective diameters of 85 and 275 µm were used. Those microcarriers were synthetized with Carroucell patented Sol-Gel process. At the end of the synthesis, microcarriers were functionalized by the inclusion of positive charges at their surface. Microcarriers sterility was obtained by autoclaving for 30 minutes at 121°C, 1.2 PSI.

Culture of tumor cells on Carroucell FlexiGrow™ microcarriers in T25 culture flasks.

An innovative 2-step culture protocol was used (Figure 2).

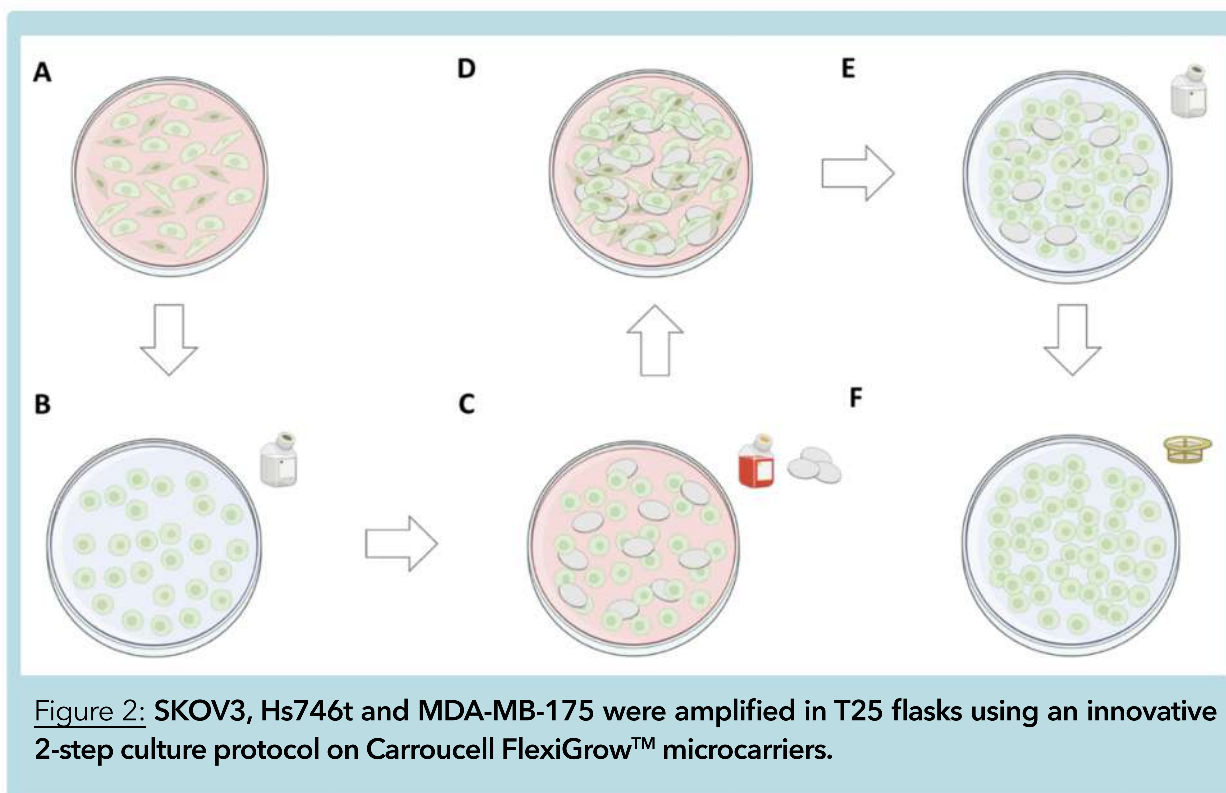


Figure 2: **SKOV3, Hs746t and MDA-MB-175 were amplified in T25 flasks using an innovative 2-step culture protocol on Carroucell FlexiGrow™ microcarriers.**

(A): 3.6 x 105 SKOV3, 3 x 105 Hs746t and 1 x 10^6 MDA-MB-175 cells were seeded in individual T25 culture treated flasks, respectively. Cells were incubated for 4, 8 and 3 days respectively at 37°C, 80% humidity, and 5% CO2. The final volume of medium in each flask was 5 mL.

(B): At the end of the culture, cells were washed with PBS and detached by a 5-minute incubation with 1 mL Trypsin –EDTA. Cell quantity and viability were quantified after Trypan Blue staining using automated cell counting (LUNA II - Logos Biosystems, South Korea).

(C) and (D): Following quantification, total SKOV3, Hs746t and MDA-MB-175 cell amount was seeded again in the same T25 flask supplemented with Carroucell FlexiGrow™ microcarriers. The ratio cells/microcarriers was set to 15 mg per 10^6 cells. Carroucell

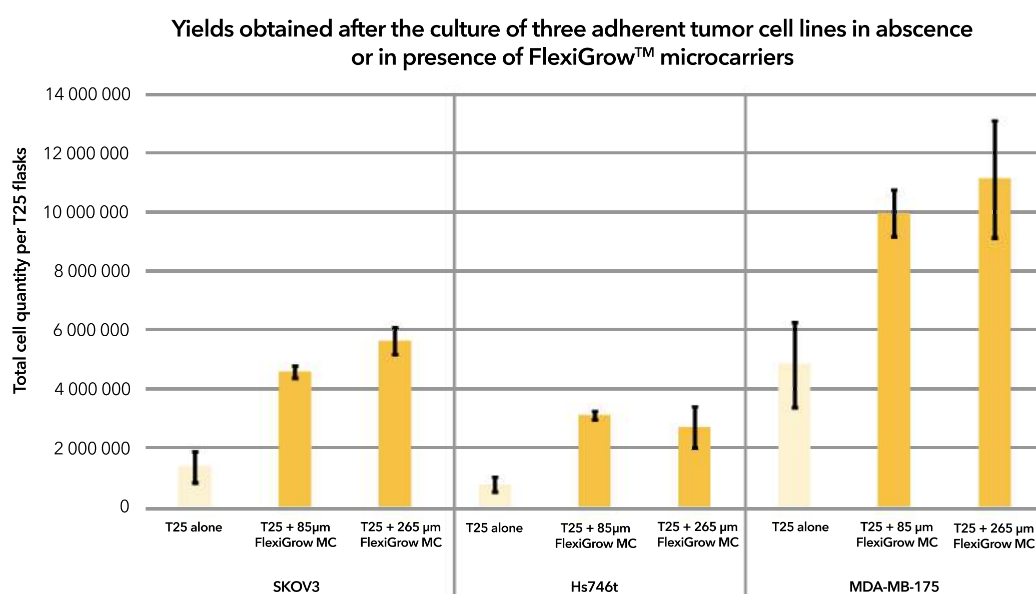
FlexiGrow™ microcarriers had diameters of 85 and 265 µm, respectively. Both SKOV3 and MDA-MB-175 cells were cultured for 4 additional days on Carroucell FlexiGrow™ microcarriers. Hs746t were cultured for 8 additional days on Carroucell FlexiGrow™ microcarriers. Final volume of medium in each flask was adjusted to 10 mL.

(E): At the end of the 2-step culture protocol on microcarriers, cells were washed in PBS and harvested by a 10-minute incubation with 2 mL Trypsin – EDTA.

(F): 4 mL of medium was added, and cells were separated from the microcarriers by filtration. 40 µm and 100 µm Corning© Cell Strainers were used for microcarriers with 85 and 265 µm diameters, respectively. Total cell quantity and viability were quantified after Trypan Blue staining and compared to the ones obtained without microcarriers.

Results and discussion

A



B

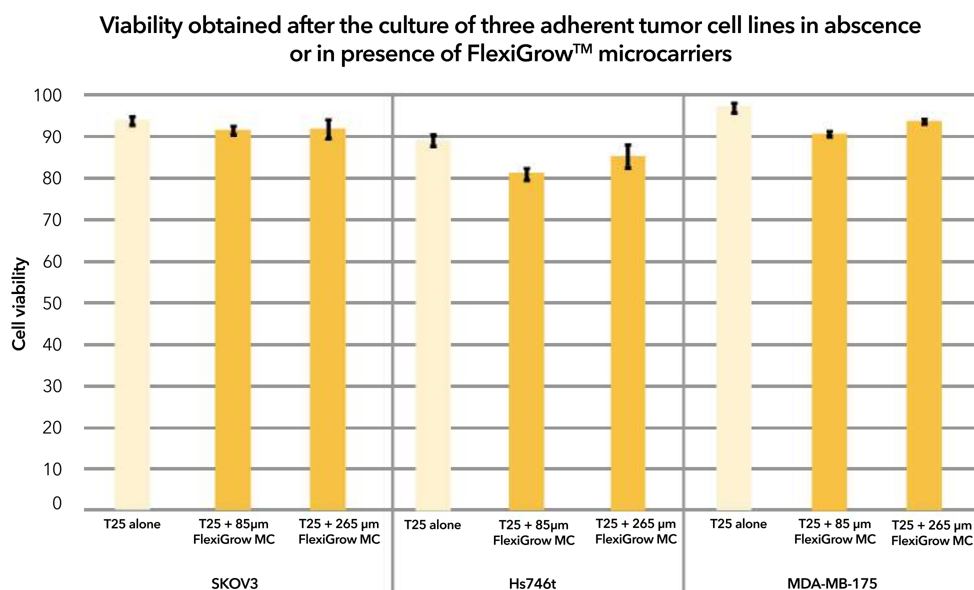


Figure 3: **Total quantity (A) and viability (B) of SKOV3, Hs746t, and MDA-MB-175 cells after their culture in T25 flasks in absence and in presence of Carroucell FlexiGrow™ microcarriers.**

CARROUCELL
Tailoring microcarriers

FlexiGrow™ Carroucell

By applying Carroucell innovative 2-step protocol for adherent cell amplification in static culture environment, 4.6 x 10^6 and 5.6 x 10^6 SKOV3 cells were obtained per T25 on 85 µm and 265 µm FlexiGrow™ microcarriers, respectively. This is 3.3 and 4.1 times more than in classic culture protocol in T25 alone.

In comparison 7.6 x 10^5 and 2.6 x 10^6 Hs746t cells were obtained in absence (T25 alone) and in presence of 265 µm FlexiGrowTM microcarriers. In presence of 85 µm FlexiGrow™ microcarriers, 3.1 x 10^6 Hs746t cells were obtained. This is 4.1 times more than in T25 alone.

Finally, 4.8 x 10^6, 9.9 x 10^6 and 11.1 x 10^6 MDA-MB-175 were obtained in absence, or in presence of 85 µm and 265 µm FlexiGrow™ microcarriers, respectively. In this last condition, 2.3 times more cells were obtained in comparison with the T25 alone.

In this study, using an innovative 2-step culture protocol, adherent tumor cells were seeded on FlexiGrow™ microcarriers after a preamplification in the culture flask alone. For each million cells that were seeded, flasks were supplemented with 15 mg of FlexiGrow™ microcarriers. This optimal cell/microcarriers ratio has been optimized by Carroucell and showed excellent results for numerous types of adherent cells. This ratio permits to significantly increase the adherence surface available for the cells while preserving an optimal culture homogeneity.

Noteworthy, the culture on FlexiGrow™ microcarriers resulted in different outcomes according to the cell types and the microcarriers diameters. SKOV3, Hs746t and MDA-MB-175 cells are three different tumor cell types, isolated from three different organs, and characterized by completely different phenotypes and functionalities. Adapting FlexiGrow™ microcarriers size is therefore a crucial strategy to:

Optimize cell interaction with microcarriers.

Optimize the formation of cell-microcarriers tridimensional structures,

Favor the optimization of the total adherence surface provided by both the culture flask and the microcarriers.

Furthermore, post-amplification on Carroucell FlexiGrow™ microcarriers, the viability of cells exceeded 80%. This noteworthy outcome attests to the compatibility and supportive nature of the microcarriers culture protocol, ensuring the preservation of cell health and functionality throughout the amplification process.

The adherence to stringent viability thresholds reaffirms the reliability and reproducibility of the Carroucell protocol. This emphasizes its potential for advancing cell culture methodologies in Oncology. More specifically for Antineo, such approaches aim to provide turn-key protocols while reducing resources dedicated to cell amplification processes and conserving the high robustness of the proposed in vivo cancer models.

Conclusions

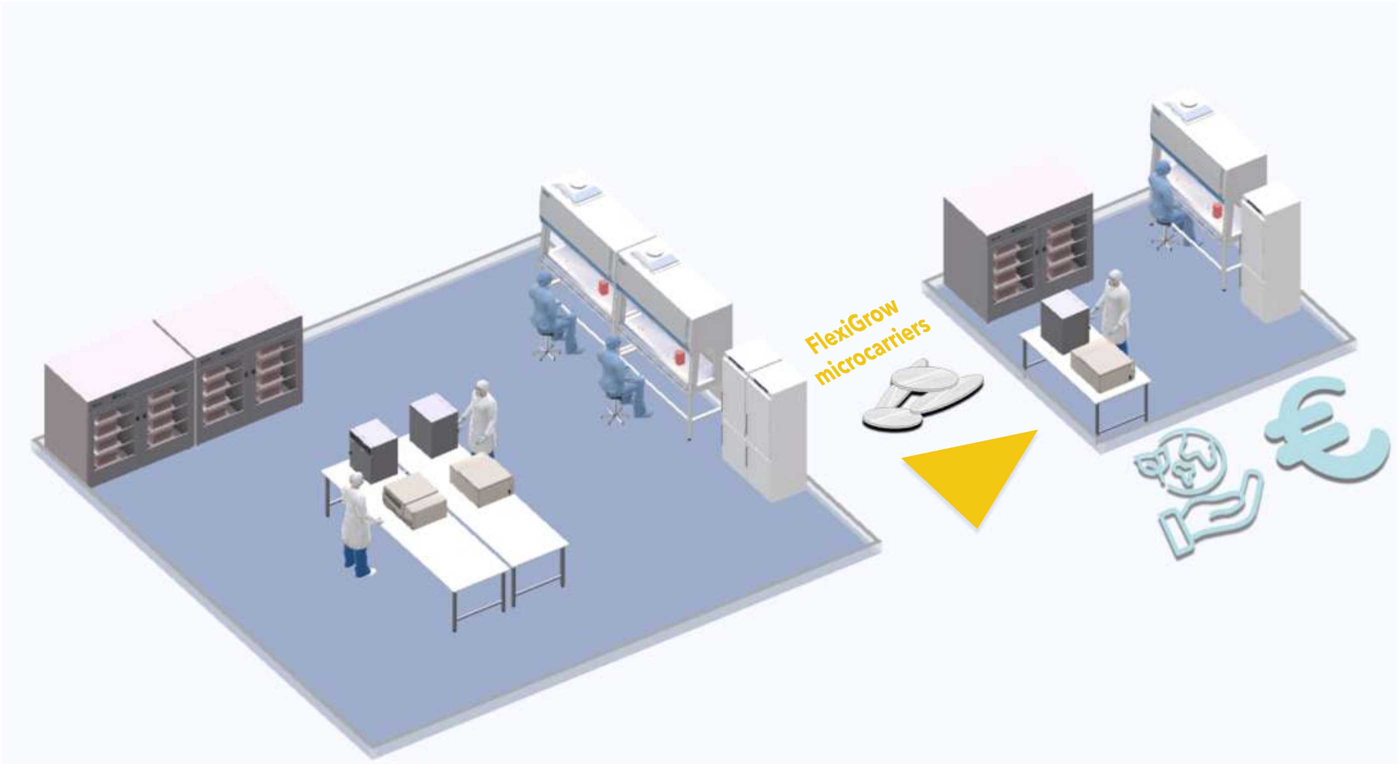


Figure 4: **Carroucell FlexiGrow™ 2-step culture protocols lead to significant advantages.**

The utilization of Carroucell's amplification process on FlexiGrow™ microcarriers initiates a transformative phase marked by significant efficiency gains. This innovative strategy ensures a 4-fold reduction in spatial requirements, a 2.5-fold decrease in consumables (encompassing media, trypsin, and plastic waste), and a 2-fold decrease in the time required by the technical team, as illustrated in Figure 4. This remarkable optimization of resources dedicated to cell culture is seamlessly achieved without disrupting established production chain practices.

Essentially, these strategic measures empower Antineo to reallocate a substantial portion of resources toward their core business: delivering resilient and versatile animal models in oncology. This streamlined operational approach not only minimizes costs but also provides Antineo with the agility and resources required to strengthen their position as pioneers in delivering cutting-edge solutions for oncology research. Carroucell's approach revolutionizes resource utilization in cell culture, ensuring unparalleled efficiency, cost-effectiveness, and steadfast support for the core objectives of esteemed partners such as Antineo.

References

[1]A. M. Nagornykh, M. A. Tyumentseva, A. I. Tyumentsev, et V. G. Akimkin, « Protocol for chronic hepatitis B virus infection mouse model development by patient-derived orthotopic xenografts », PLoS ONE, vol. 17, no 2, p. e0264266, févr. 2022, doi: 10.1371/journal.pone.0264266.

[2]V. Forest, L. Campos, M. Péoc'h, D. Guyotat, et J.-M. Vergnon, « [Development of an experimental model for the study of the effects of cryotherapy on lung tumours] », Pathol. Biol. (Paris), vol. 53, no 4, p. 199�203, mai 2005, doi: 10.1016/j.patbio.2004.08.002.

[3]J. Jung, H. S. Seol, et S. Chang, « The Generation and Application of Patient-Derived Xenograft Model for Cancer Research », Cancer Res. Treat. Off. J. Korean Cancer Assoc., vol. 50, no 1, p. 1�10, janv. 2018, doi: 10.4143/crt.2017.307.

[4]A. S. T. Smith, J. Macadangdang, W. Leung, M. A. Laflamme, et D.-H. Kim, « Human iPSC-derived cardiomyocytes and tissue engineering strategies for disease modeling and drug screening », Biotechnol. Adv., vol. 35, no 1, p. 77�94, janv. 2017, doi: 10.1016/j.biotechadv.2016.12.002.

[5] le Clainche T, Moisan A, Coll JL, Martel-Frachet V (2021) The disc-shaped microcarriers: a new tool for increasing harvesting of adipose-derived mesenchymal stromal cells. Biochem Eng J 174:108802. doi: 10.1016/j.bej.2021.108082