Integrity™ Xpansion™ Multiplate Bioreactor: The Scalable Solution for Adherent Stem Cell Expansion

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Introduction

Adherent stem cell amplification processes use traditional polystyrene T-flasks or multitray stacks. Such culture methods are not suitable for large-scale production since they involve multiple manual operations and large numbers of recipients and culture rooms. Capital and operating costs for large-scale production are also prohibitive. To date, implementation of other accepted approaches for large-scale stem cell production, such as microcarriers and scaffold bioreactors, has been impeded by cell sensitivity to shear stress and harvesting issues.

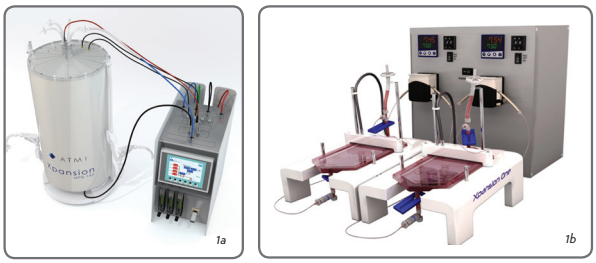

Figure 1: Description of the (a) Xpansion Multiplate Bioreactor (180 plates: surface = 115 000 cm², working volume = 19 L) and (b) Xpansion-One Bioreactor (culture surface = 128 cm², working volume = 20ml) with their controllers.

In response to the lack of suitable large-scale expansion and recovery systems for adherent stem cells, ATMI LifeSciences has developed a new 2-D bioreactor, the Integrity™ Xpansion™ Multiplate Bioreactor. Due to its large surface area of up to 115 000 cm² and multiplates design (*Figure 1a*), the system enables production of large amounts of cells in a process easily adapted from traditional T-flask or stacked-tray methods. A scaled-down version of the bioreactor, the Xpansion-One system (*Figure 1b*), has been developed to allow benchtop testing.

Here we present characteristics of the Integrity Xpansion Multiplate Bioreactor – homogeneity of cell distribution on plates and culture parameters regulation (DO and pH) – and a case study of cardiac-lineage committed cells cultures.

Bioreactor Design

The Xpansion bioreactor was designed to enable adherent cell growth in the same conditions and surfaces than in T-flasks. Cells adhere and grow on the stacked polystyrene plates. DO and pH are controlled by equilibration of media with a gaseous phase where concentration of O_2 and CO_2 is controlled. The gases diffuse through the wall of very thin silicon tubing placed in the central column. Media circulation is generated by a centrifuge pump controlling the flow rate to adapt it to appropriated shear stress requirements (*Figure 2*). The Xpansion-One (small scale model) system was designed to mimic conditions applied on cells attached on one plate of the large-scale Xpansion system. Computational fluid dynamic simulations and validations made on Xpansion system prototypes have demonstrated that media circulation through all plates is evenly-distributed. It also demonstrated that linear speed is constant between the plates and most of the plate area. Shear stress on walls was simulated on both Xpansion system scales and results showed that maximum shear stress on wall surface was inferior to 10mPa for an average linear speed of 2mm.s^{-1} (not shown).

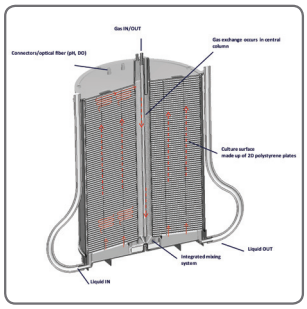

Figure 2: Description of Xpansion bioreactor and schematic view of media circulation through Xpansion system plates.

Xpansion-One

Mesenchymal stem cells from bone marrow (BMSC) were expanded, committed to cardiac-lineage (BMSC-CCL) and frozen. These committed cells were used to validate the biocompatibility of the Xpansion Multiplate Bioreactor (not shown) and amplified in the Xpansion-One system vs. T-flask. Population Doubling Time obtained was equivalent in both systems (*Figure 3*). Microscopic observation enabled us to follow the confluence efficiency (not shown) and to confirm that morphology was equivalent in T-flask and the Xpansion-One system.

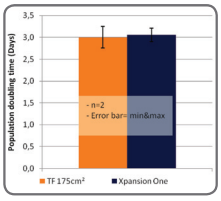

Figure 3: Comparison of Population Doubling Time (PDT) of cardiac-lineage committed mesenchymal stem cells cultivated in TF175 cm² and in Xpansion One system. Cells were grown in the same conditions: one week duration, same ratio $V_{media}/S_{culture}$, no pH and DO controls.

Following cardiac lineage commitment, levels of the nucleic form of the transcription factor of MEF2C in cells grown in the Xpansion-One system were at least equivalent to those obtained in cells in T-flasks (*Figure 4*). In addition, expression levels of specific markers of adipogenic, oesteogenic and chondrogenic differentiation remained low and equivalent in both culture systems.

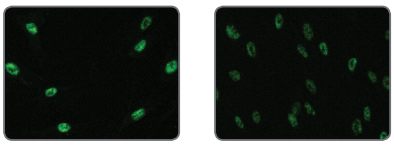

Figure 4: Comparison of MEF2C transcription factor location profile between cardiac-lineage committed mesenchymal stem cells cultivated in Xpansion-One (a) and TF (b). MEF2C transcription factor is mainly located in the cell nuclei upon cardiac lineage commitment.

Integrity Xpansion

Cultures of BMSC-CCL and Vero cells have been performed in the Integrity Xpansion Multiplate Bioreactors (Xpansion-MPB) with 10 and 50 plates. Cultures of Vero cells in Xpansion-MPB-10 indicated that regulations of pH and DO using a thin silicone tubing sustains at least up to 118 000 Vero cells/cm² (*Figure 5*). Hence, we do not expect regulation problems with any kind of stem cells (because cell density achieved by stem cells is usually less than by Vero cells). Cell expansion performed in Xpansion-MPB-10 and -50 plates indicated that Vero cells and BMSC-CCL colonized bioreactor plates (Figure 6, 7). Homogeneity of cell distribution on all bioreactor's plates was instigated in Xpansion-MPB-50 using Vero cells. This culture indicates cells colonization on all plates of the reactor (Figure 7); the three top plates of Xpansion-MPB-50 can be observed using a LED microscope (developed by Ovizio, Belgium). At the end of the culture, cells were fixed and colored by crystal violet and homogeneity of the reactor was observed by microscopy. All plates were colonized by cells with similar confluencies.

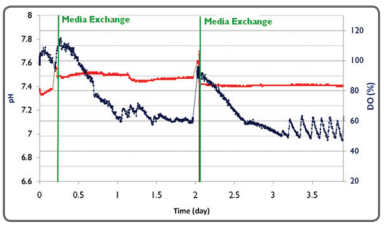

Figure 5 (above): Time course of pH (red line) and DO (blue line) in the Vero cells culture in Xpansion. Xpansion-10: Cells were inoculated at 45 000 cells/cm², culture duration was four days. set-point = pH > 7.4 and DO > 50%.

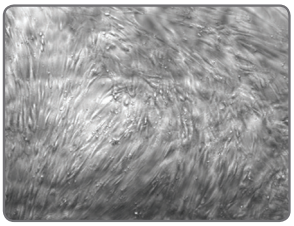

Figure 6: Mesenchymal stem cells commited to cardiac-lineage colonization of Xpansion system. Magnification = X40. Xpansion-10: Cells were inoculated at 2500 cells/cm², culture duration was one week, set-point = pH > 7.3 and DO > 50%.

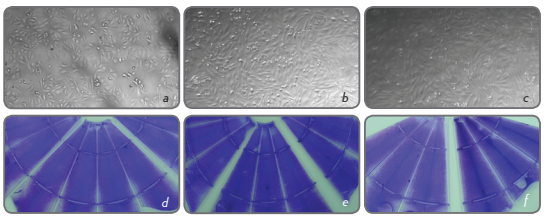

Figure 7: Colonization of Xpansion-50 plates by Vero cells after 6h (a), 24h (b) and 48h (c). Magnification = X40. Pictures of plates #7 (d), 27 (e) and 47 (f) at the end of the culture. Xpansion-50: Cells have been inoculated at 45 000cells/cm², culture duration was two days, numbers of plates from top to bottom.

Conclusion

CFD studies and experimental validations on prototypes demonstrated that Xpansion Multiplate bioreactors generate a very gentle laminar flow. Maximum wall shear stress does not exceed 10mPa which is 100 to 1000 times less than stirred-tank bioreactors.

The BMSC-CCL amplification case study proved the Xpansion-One system is an efficient solution for cell amplification and maintains cell differentiation profiles. Cell confluence, population doubling time, morphology and cell differentiation were equivalent to T-flasks cultures.

BMSC-CCL and Vero cells cultures indicated that pH and DO regulations using a thin silicone tubing can sustain high cell densities. Cells attach, spread and colonize all plates of the Xpansion Multiplate bioreactors. All plates are colonized in the same way.

A specific LED microscope developed by Ovizio (Belgium) allows, at least, the observation of the three top plates of Xpansion-10 and -50 plates (respectively 6 360 and 31 800 cm² available for cells, additionally, light is able to travel through all of the Xpansion-10 and -50 plates). Cell density and morphology are monitored in Xpansion-MPB-180 via specific Holographic microscope technology (developed by Ovizio and integrated in a close partnership with ATMI) that can show images of at least the upper six plates.