SARTORIUS

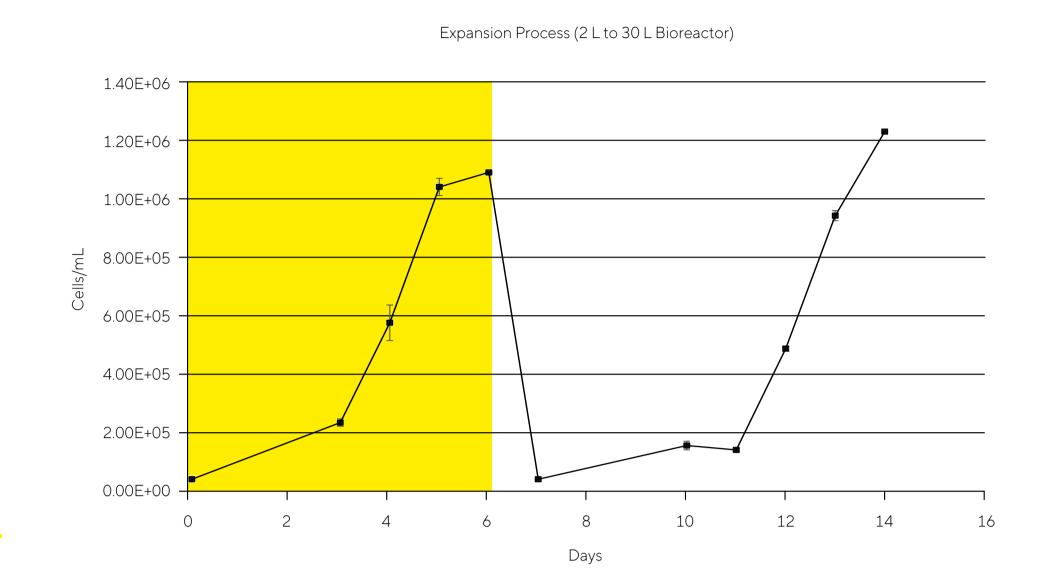
A Robust, Single-Use Solution for Expansion of Human Mesenchymal Stem Cells in Xeno-Free Medium

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Experimental Approach

The objective of this experiment was to expand umbilical cord-derived mesenchymal stem cells (MSCs) on collagen-coated microcarriers (MCs). A seed train, which consisted of a N-1, 2 L Univessel® Glass bioreactor (BR), was used to generate cells to seed a Biostat STR® 50 BR at 30 L. Cells were expanded for 7 days prior to harvest and concentration using the Ksep® 400 centrifuge.

Current Process Proposed Process Workflow An onerous planar culture process generating a limited number of cells Sufficient process for Phase I and Ib clinical trial with 20 patients Not enough to satisfy Phase II requirements of 80 patients Phase III patient cohort projected at 300 patients Phase III patient cohort projected at 300 patients

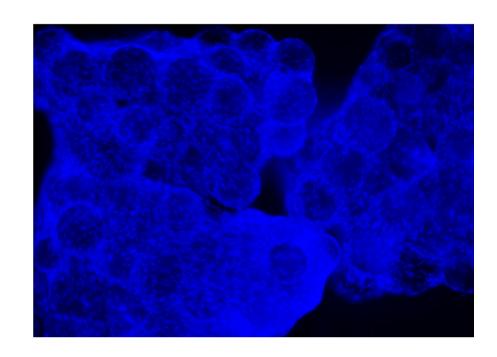


Materials









2 L Univessel®

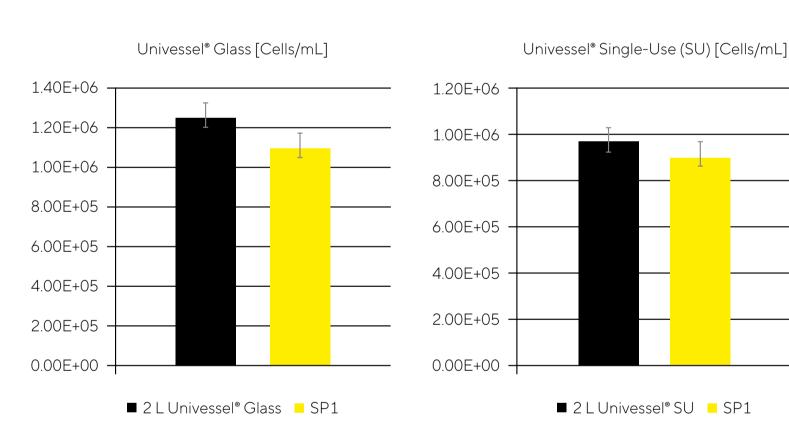
DAPI Images

Biostat STR® 50

Figure 1: Schematic of the Sartorius Pilot and Scale-up Solutions, Including, Biostat® B and Universel® Glass Bioreactors, MSC NutriStem® XF Medium, Biostat STR® 50 Gen3 Bioreactor, Ksep® 400, Solohill® Collagen-Coated Microcarriers in Microcarrier Delivery Systems (MDS), Biosealer®, Biowelder® and iQue® 3 Flow Cytometer

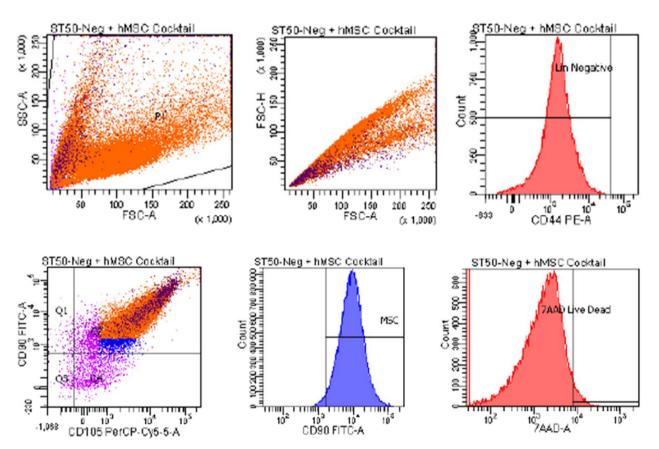
Results

Optimization of 2 L Bioreactor Process



Parameter	Target	Result	
		Glass	SU
Harvest density [Cells/mL]	≥ 6e5	1.3e6	9.7e5
Viability [%]	≥90	96	97
Harvets efficiency [%]	≥ 90	≥ 95	≥ 95
Total cell harvested	≥ 1.0e9	1.9e9	1.6e9

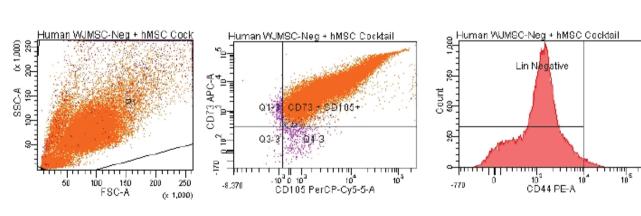
Characterization of Cells Harvested From the Biostat STR® 50 Bioreactor



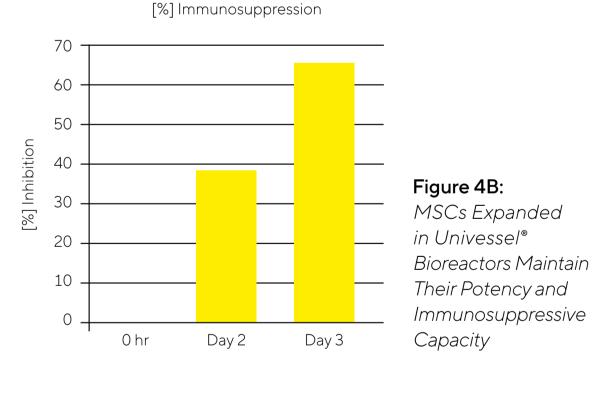
MSC Characterization (Specifications)	Total [%]
Purity (≥ 80%)	≥ 96.9
MSC characterization (≥ 95% CD73, CD90, CD105)	98
Lin negative (≤ 2% CD34, CD45, CD11b, CD19, HLA-DR)	2
Viability (≥ 80%)	97

Figure 4A: MSC Surface Markers Are Maintained After Expansion In 3D Suspension-Based Culture Systems

Characterization of Cells Harvested From Biostat® B 2 L Bioreactors



C Cocktail		
	MSC Characterization (Specifications)	Total [%]
	Purity (≥ 80%)	94
	MSC characterization (≥ 95% CD73, CD90, CD105)	97.6
-	Lin negative (≤ 2% CD34, CD45, CD11b, CD19, HLA-DR)	0.7
hanne I	Viability (≥ 80%)	91.6
10 ⁴ 10 ⁸		



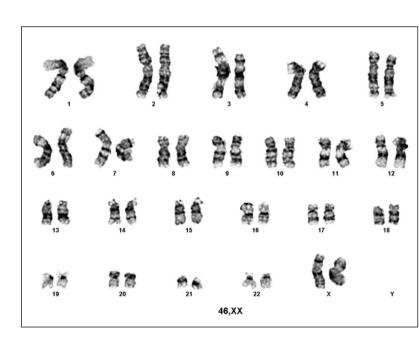


Figure 4C:
MSCs Retain
Normal
Karyotype After
Expansion in
Univessel®
Bioreactors

Figure 2A: MSC Surface Markers Are Maintained After

Expansion in 3D Suspension-Based Culture Systems

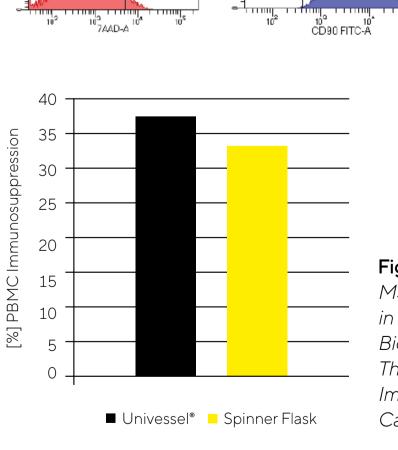


Figure 2B:
MSCs Expanded
in Univessel®
Bioreactors Maintain
Their Potency and
Immunosuppressive
Capacity

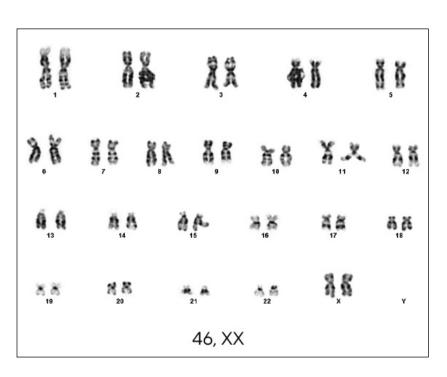
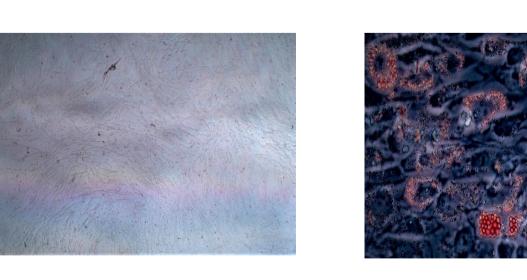
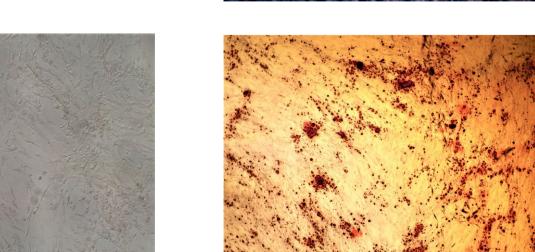


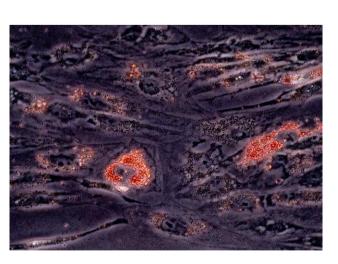
Figure 2C:
MSCs Retain
Normal Karyotype
After Expansion
in Univessel®
Bioreactors

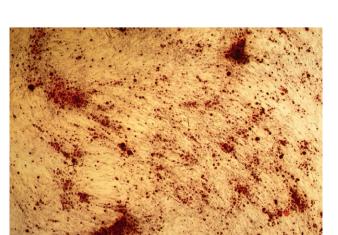
Multilineage In Vitro Differentiation of MSCs: Osteocyte and Adipocyte Lineages





2 L Univessel®



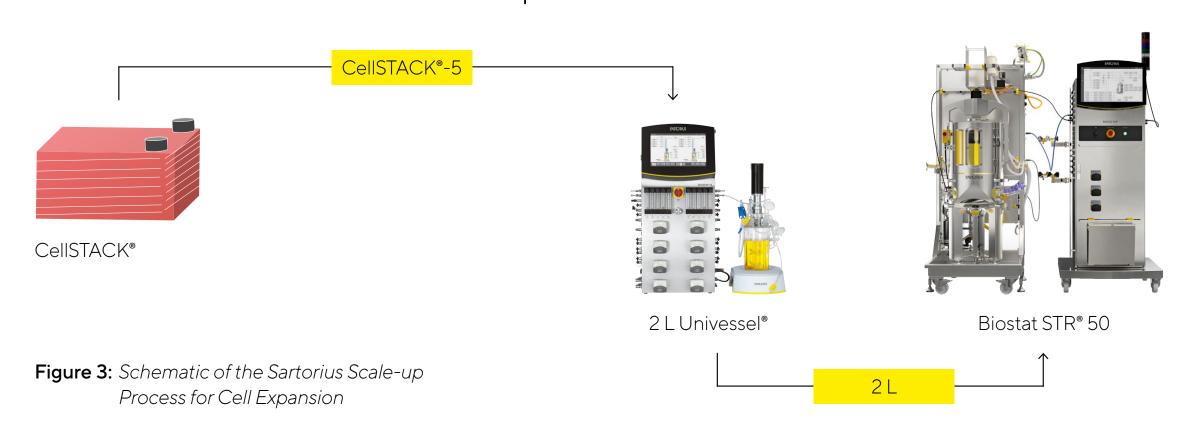


Biostat STR® 50

Osteocyte

Adipocyte

Performance of the Scale-Up Process



Summary

Control

- The Sartorius MSC pilot solution, along with the subsequent MSC manufacturing solution, comprise a scalable offering that provides continuity and enables seamless process transfer for industrialized expansion of adherent cell types
- This study provides proof of concept for the successful transition from a 2D static to a 3D suspension culture process for cell expansion, using Sartorius MSC solutions
- With these solutions, MSCs can be robustly expanded to large working volumes in a simplified seed train. Cells maintain high viability and stem cell relevant critical quality attributes
- Sartorius solutions enabled KUMED to generate enough cells to reach clinical trial goals
- This accelerates time to market and saves on production costs, ultimately leading to increased accessibility and affordability of these life-saving therapies