

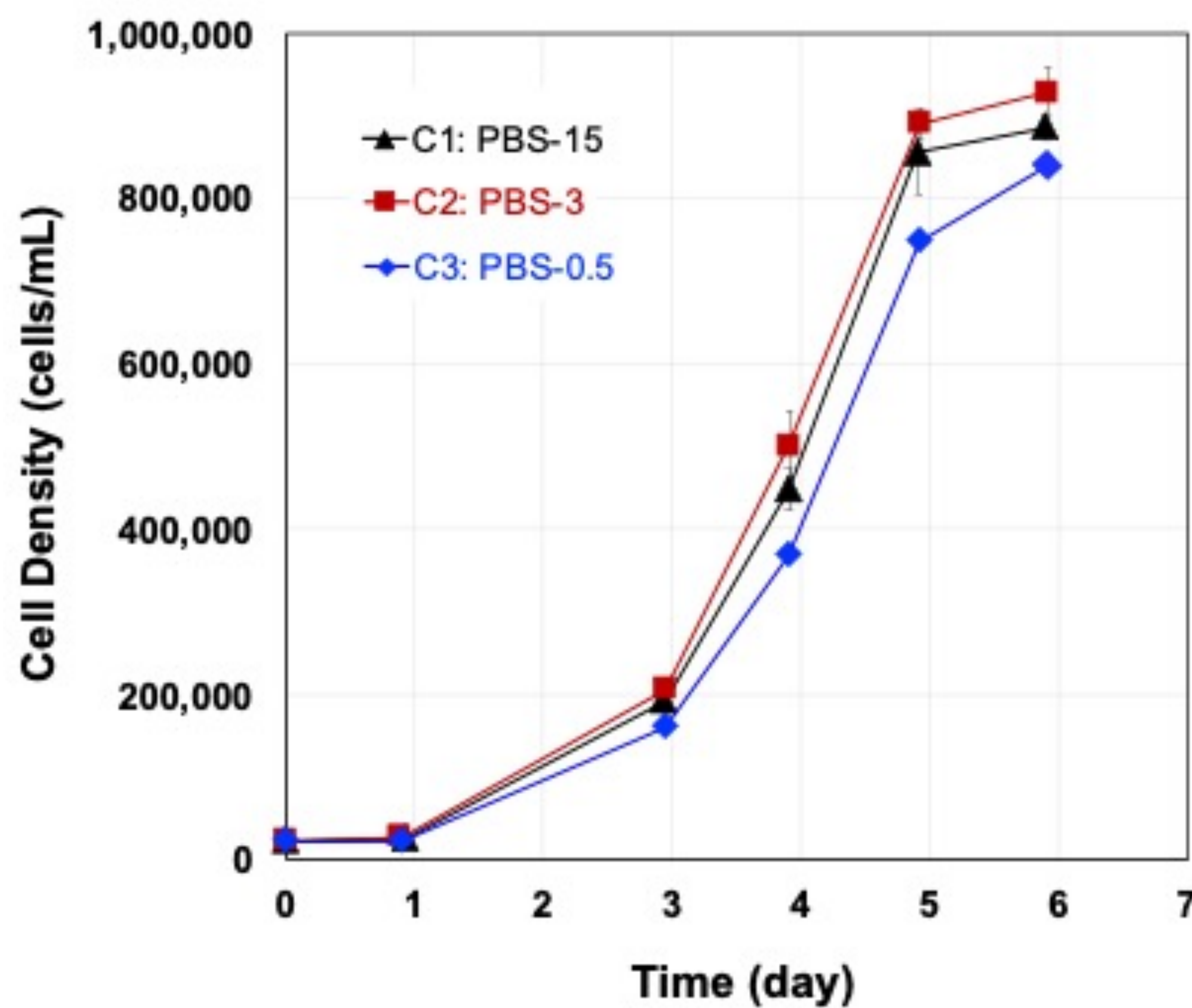
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Human Mesenchymal Stem/Stromal Cells (hMSCs) Grown On The Surface Of Microcarriers In Vertical-Wheel Bioreactors

Figure 1: Scalable Expansion Of hMSCs On Microcarriers In Vertical-Wheel Bioreactors



Initial Densities

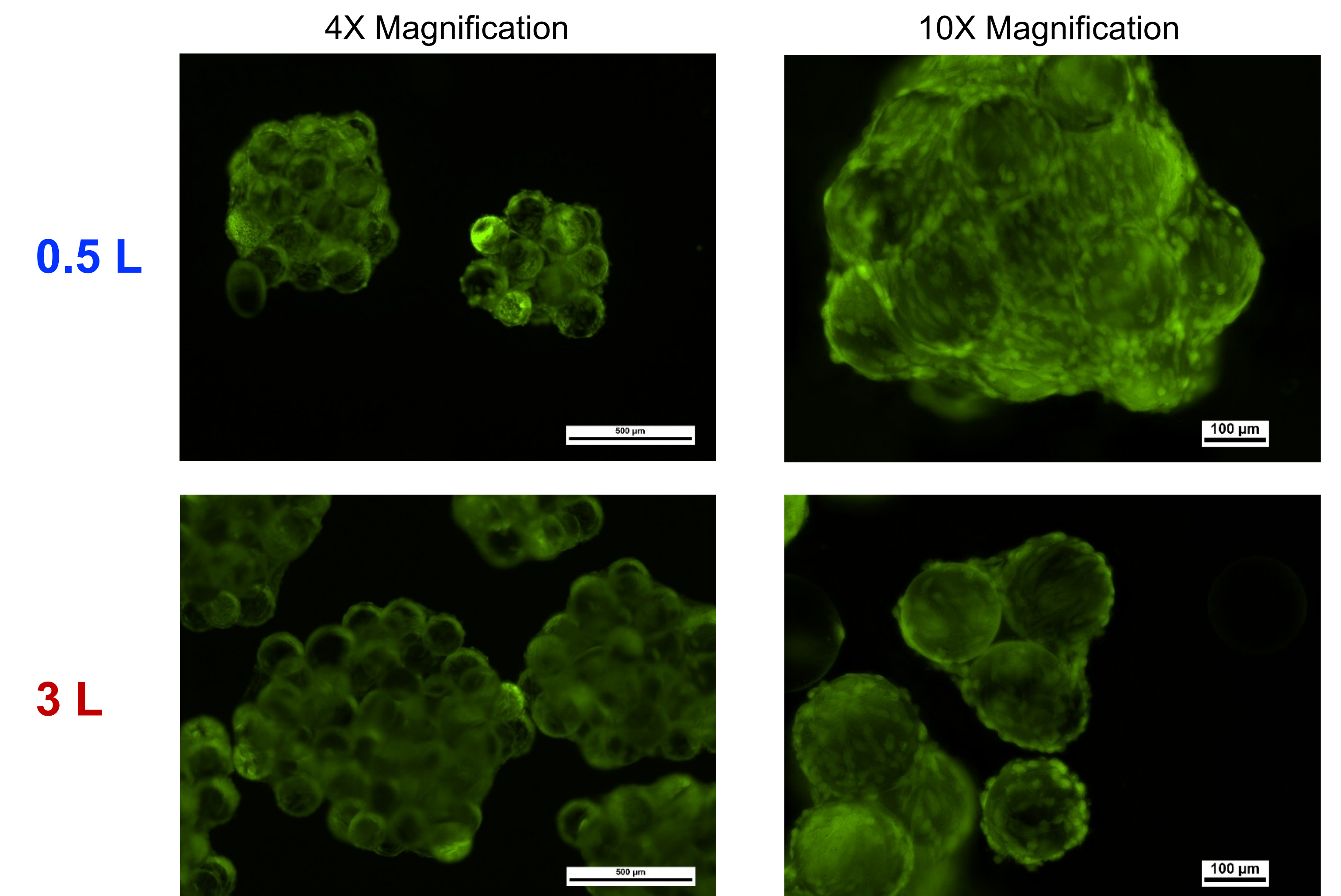
Cell inoculation: 22,500 cells/mL
 Microcarriers: 20 g/L

Agitation Rates (RPM)

	0.5 L	3 L	15 L
Days 0-3	27	18	12
Days 3-4	29	20	15
Days 4-6	38	25	17

- ❖ Cell densities >800,000–900,000 cells/mL consistently achieved after six days at three different bioreactor scales, with over 10 billion viable hMSCs produced in a single 15 L bioreactor → further process optimization possible
- ❖ Cell recovery efficiency ~86% at 15 L scale (includes in-vessel dissociation, harvest, wash, and concentration)

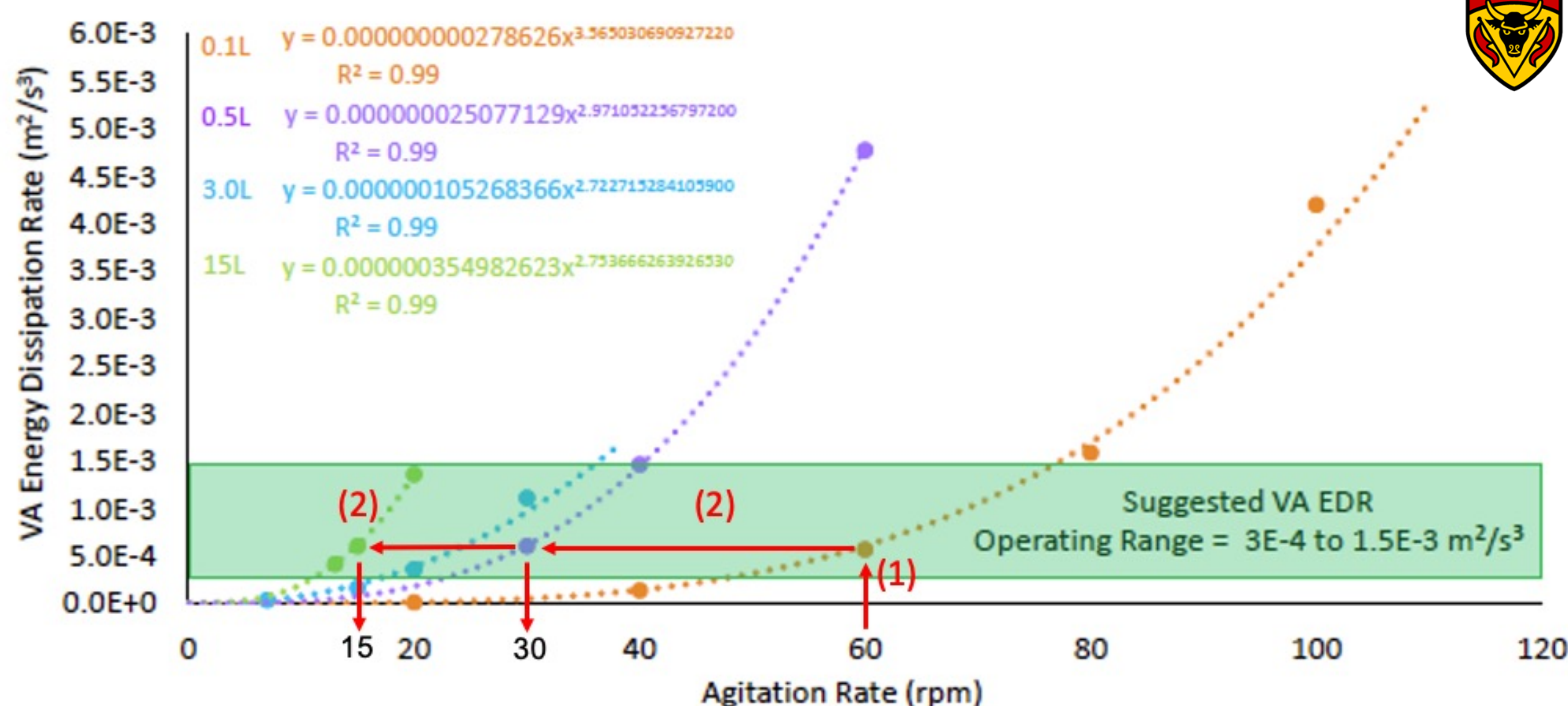
Figure 2: Photomicrographs Of hMSCs On Surfaces Of Microcarriers, Day 5



- ❖ hMSCs (light green spots) grown on the surfaces of spherical microcarriers (darker circles) and harvested on days 5 and 6 passed quality assays

Human Induced Pluripotent Stems (hiPSCs) Grown As Cell Aggregates In Vertical-Wheel Bioreactors

Figure 3: VA EDR vs. Agitation Curves For Different Scales Of Vertical-Wheel Bioreactors



- ❖ Computational fluid dynamics modeling used to calculate volume average energy dissipation rate (VA EDR) for various combinations of bioreactor scales and agitation rates → VA EDRs plotted to generate best-fit curves
- ❖ Example of how VA EDR can be used as a predictive tool to minimize guesswork during process scale up:
 - 1) At small scale (0.1L), determine the RPM that yields optimal aggregates (60 rpm) → the corresponding VA EDR is the "target VA EDR" (6.1E-4 m²/s³)
 - 2) Find the target VA EDR on larger scale curves → corresponding agitation rate will produce similarly optimal aggregates (30 rpm @ 0.5 L and 15 rpm @ 15L)
- ❖ Results from multiple experiments with various collaborators: uniformly spherical hiPSC aggregates achieved at various scales and agitation rates when VA EDR falls within suggested range of 3.0E-4 to 1.5E-3 m²/s³

Figure 4: Correlation Between VA EDR And hiPSC Aggregate Morphology

Volume (L)	Agitation (RPM)	VA EDR (m²/s³)	CFD Model (EDR)	Day 1 (10x)	Day 3 (10x)	Day 5 (10x)	Day 7 (10x)
0.1	60	6.1E-4					
0.5	18	1.4E-4					Clumped cell mass too large to suspend
0.5	30	6.1E-4					
0.5	40	1.4E-3					
0.5	60	4.8E-3					

- ❖ 0.1 L, 60 RPM → uniformly spherical aggregates, with target VA EDR of 6.1E-4 m²/s³ (within range)
- ❖ Target VA EDR matched at 0.5 L, 30 RPM → by day 7, aggregates are identical to those at 0.1 L scale
- ❖ 0.5 L, 40 RPM: VA EDR near upper limit of range → aggregates still spherical but slightly smaller
- ❖ 0.5 L, 18 RPM & 0.5 L, 60 RPM: VA EDRs outside of range → highly variable aggregate morphologies