Scale-up study for ex-vivo expansion of allogeneic natural killer cells in stirred-tank bioreactor

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Process development for large-scale *ex vivo* expansion of allogeneic natural killer cells in stirred-tank bioreactor

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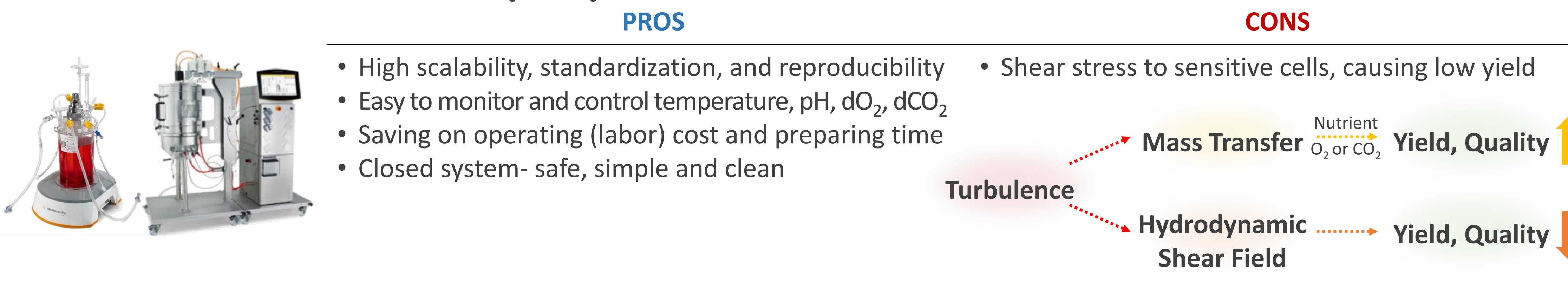** Corresponding author/ e-mail: shpaik@greencross.com*
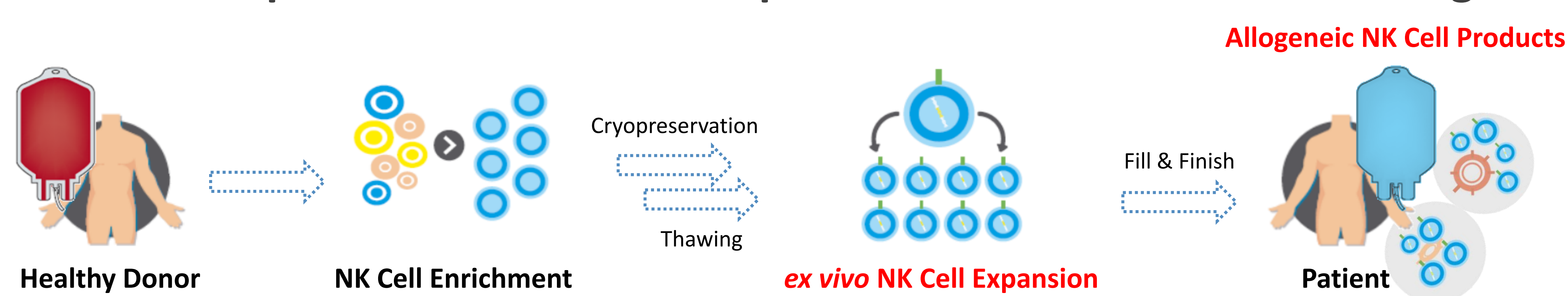
INTRODUCTION

1. Natural Killer (NK) Cell

Natural killer (NK) cells are a type of lymphocytes in the blood that are responsible for innate and adaptive immune response and they mature in the liver and bone marrow. Being a key role in host defense system with direct and indirect killing of virus-infected cells or cancer cells, NK cell has been considered an attractive candidate for cancer therapy. Peripheral blood shows the low frequency of NK cells, so the *ex vivo* expansion method is important to obtain sufficient NK cells for therapeutic use.

2. Stirred-Tank Reactor (STR)

PROS	CONS
• High scalability, standardization, and reproducibility	• Shear stress to sensitive cells, causing low yield
• Easy to monitor and control temperature, pH, dO_2, dCO_2	
• Saving on operating (labor) cost and preparing time	
• Closed system- safe, simple and clean	

Turbulence → Mass Transfer (Nutrient, O_2 or CO_2) → Yield, Quality ↑

Turbulence → Hydrodynamic Shear Field → Yield, Quality ↓

3. Schematic Representation of *ex vivo* Expansion of NK Cells at Manufacturing Scale

Allogeneic NK Cell Products

Healthy Donor → NK Cell Enrichment → Cryopreservation / Thawing → *ex vivo* NK Cell Expansion → Fill & Finish → Patient

Stirred-tank bioreactor could be considered as an optimal alternative system for large-scale NK cell expansion compared with other ones because it is automated, less labor-intensive, scalable, well-controlled and cost-effective. We firstly developed a lab-scale bioreactor process for *ex vivo* expansion of NK cell in the world. With the need for reduction in production cost and easy-to-control large-scale production, we advanced the lab-scale bioreactor process to manufacturing scale by 50 L.

4. Aim : Manufacturing Scale-Up with Comparability to Lab Scale Bioreactor

METHOD

Comparability between Different Scale Bioreactors in *ex vivo* NK Cell Expansion

Lab-scale Bioreactor (2 L) → Scale-up → Manufacturing-scale Bioreactor (50 L)
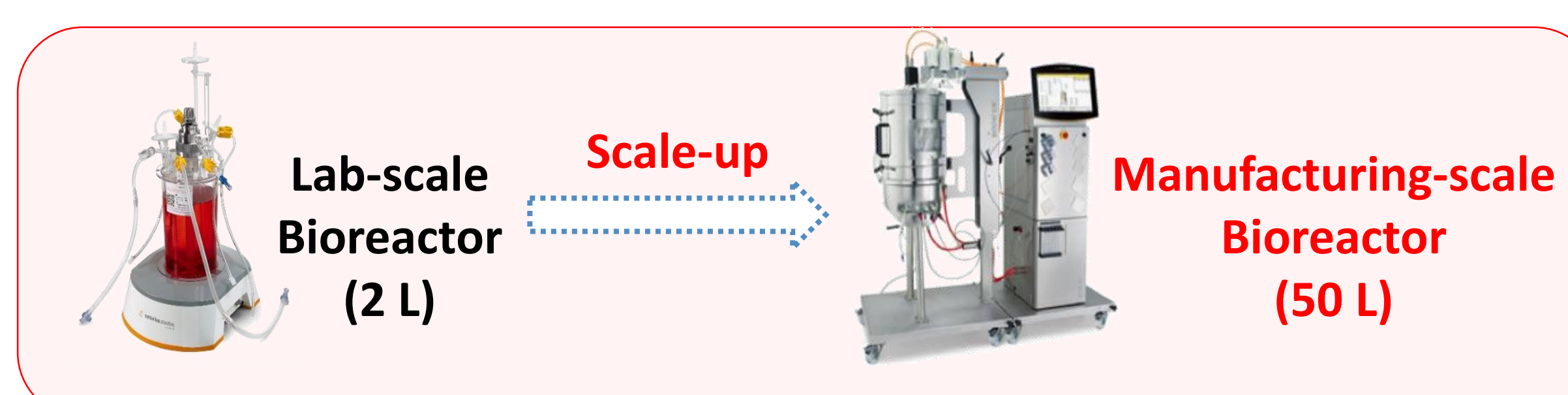
1. Engineering Parameters for Scale-Up

• **Classical Parameters**
- Geometry of Bioreactors
- Power Input (P/V) Work on Mixing

$$\frac{P}{V} = \frac{Ne \cdot \rho_L \cdot N^3 \cdot D^5}{V}$$

• **Advanced Parameters**
- Shear Stress

$$\tau = \mu \frac{\delta u}{\delta y}$$

- Mass Transfer

$$k_L = C_1 \cdot \sqrt{D_L} \cdot \left(\frac{\varepsilon_L}{v_L}\right)^{0.25}$$

Latin symbols
C_1 Constant (for Oxygen Diffusivity)
D Diameter of Stirrer
D_L Oxygen Diffusivity (1.98 × 10^{-9} m^2 s^{-1} in water at 20°C)
k_L Oxygen Transfer Coefficient
M Torque
N Rotation Speed
Ne Newton Number
P Power
u Velocity to x-axis
V Volume

Greek symbols
ε_L Turbulent Energy Dissipation Rate
μ Dynamic Viscosity
v_L Kinematic Viscosity
ρ_L Density of Liquid Phase
τ Shear Stress

2. Computational Fluid Dynamics (CFD)

• ANSYS Software Student Ver. 19.2
• General Turbulent Model: k-ε Model

3. In-Process Control (IPC) & Quality Control (QC)

• Viable Cell Density (VCD)
• Viability
• Concentration of Glucose, Lactate
• Potency (Cytotoxicity)
• Identity
• Purity

It is necessary to provide homogenous culture conditions. So we studied effects of agitation at manufacturing-scale (50 L) and figured out an optimum condition based on the comparability to the lab-scale (2 L) bioreactor, for which In-Process Control (IPC) and Quality Control (QC) were carried out in terms of growth rate, viability cytotoxicity, and purity.

CLASSICAL PARAMETERS CORRELATION (2 L AND 50 L STR)

Basic parameters including two dimensionless numbers, power input (P/V) and Newton number (Ne) are applied to scale up bioreactor process. P/V_{2L} and Ne_{2L} were calculated based on the optimal condition where NK cells were well cultivated at lab-scale bioreactor (2 L). The same values of them at manufacturing scale (50 L) (P/V_{50L} and Ne_{50L}) can determine agitation speed (RPM) in 50 L bioreactor process. This scale-up process could be expected to show comparable performance compared with 2 L scale.

ADVANCED PARAMETERS CORRELATION (2 L AND 50 L STR)

Computational Fluid Dynamics (CFD) simulation has become a widely applicable numerical technique for understanding local properties (e.g., fluid velocity, gas holdup, and shear stress). Navier-Stokes equation is basic for calculating flow dynamics with time, and its derived modeling relating to turbulence is introduced to predict advanced parameters. Shear strain rate (SSR) and turbulent kinetic energy (TKE) are the most two well-known parameters to study cell damage in cultivation and mass transfer, respectively.

Applying calculated RPM to operation at 50 L scale, x, y, z-axis velocities (u, v, w) were 1.83, 1.84, 1.18-fold faster than in 2 L scale, respectively (**Fig. 1**). Distribution of faster velocity generally causes the shear field to become intense, which can raise the issues in cell damage during cultivation. CFD results, however, showed the reduction in shear strain rate by 37.4% in scaling up to 50 L (**Fig. 2**), and turbulent kinetic energy (TKE) of 50 L bioreactor got more increased by 47.3% along Z-axis than of 2 L bioreactor (**Fig. 3**). Both macroscopic similarity and infinitesimal analysis via CFD demonstrated that scale-up to 50 L could lower SSR and enhance TKE with the comparability to 2 L scale bioreactor process, which could be available to cultivate NK cells.
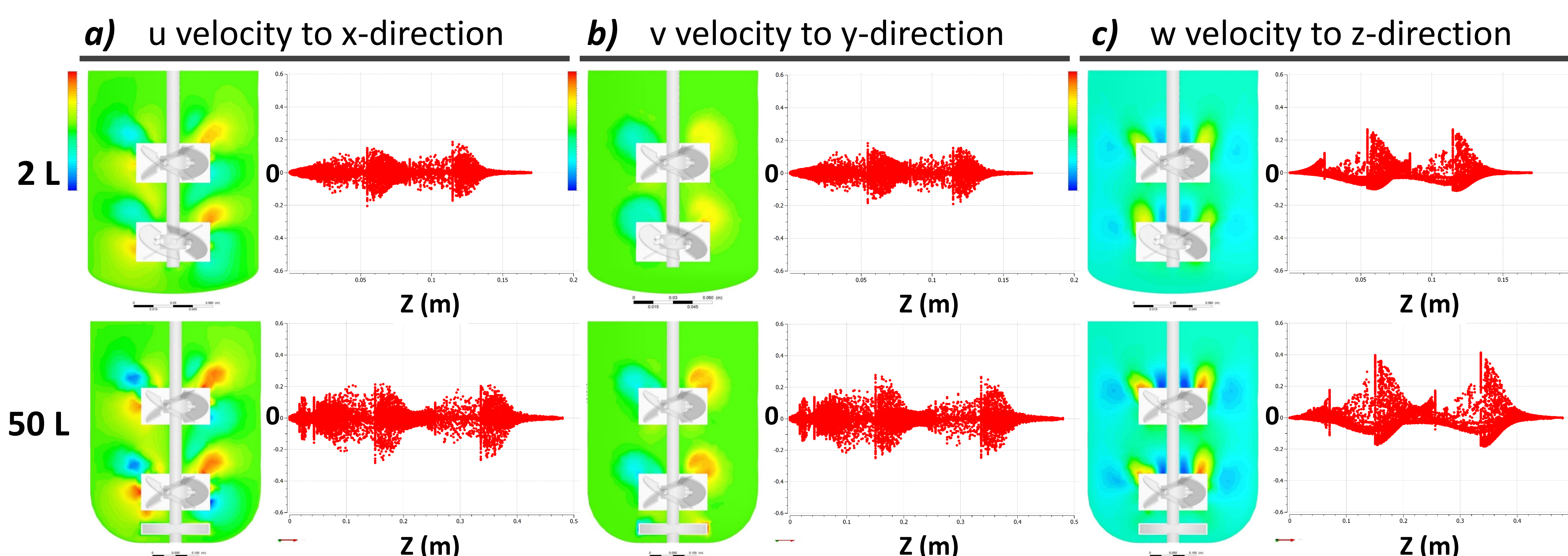

Figure 1. Comparison of velocity distribution on coordinate frame (x-, y-, z-axis) at lab-scale (2 L) and manufacturing-scale (50 L). Contour graphs on xz-plane (left) and scatter graphs (right) on z-axis represented *a)* u velocity (x-direction) at 2 L scale and 50 L scale (ave. 0.011 m s^{-1} and ave. 0.020 m s^{-1}, respectively), *b)* v velocity (y-direction) at 2 L scale and 50 L scale (ave. 0.011 m s^{-1} and ave. 0.020 m s^{-1}, respectively), and *c)* w velocity (z-direction) at 2 L scale and 50 L scale (ave. 0.029 m s^{-1} and ave. 0.034 m s^{-1}, respectively)
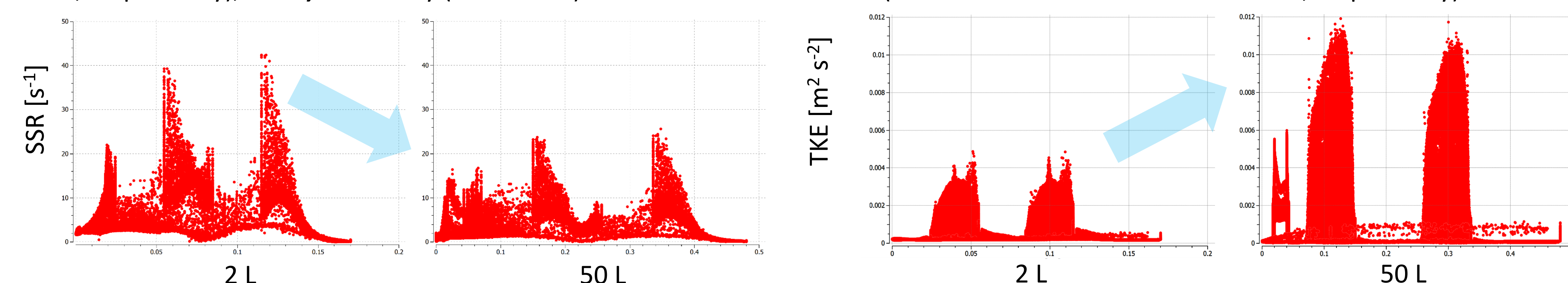

Figure 2. Shear strain rate (SSR) at 2 L scale and 50 L scale (5.98 s^{-1} and 3.74 s^{-1} respectively)

Figure 3. Turbulent kinetic energy (TKE) at 2 L scale and 50 L scale (0.82 J kg^{-1} and 1.20 J kg^{-1}, respectively)
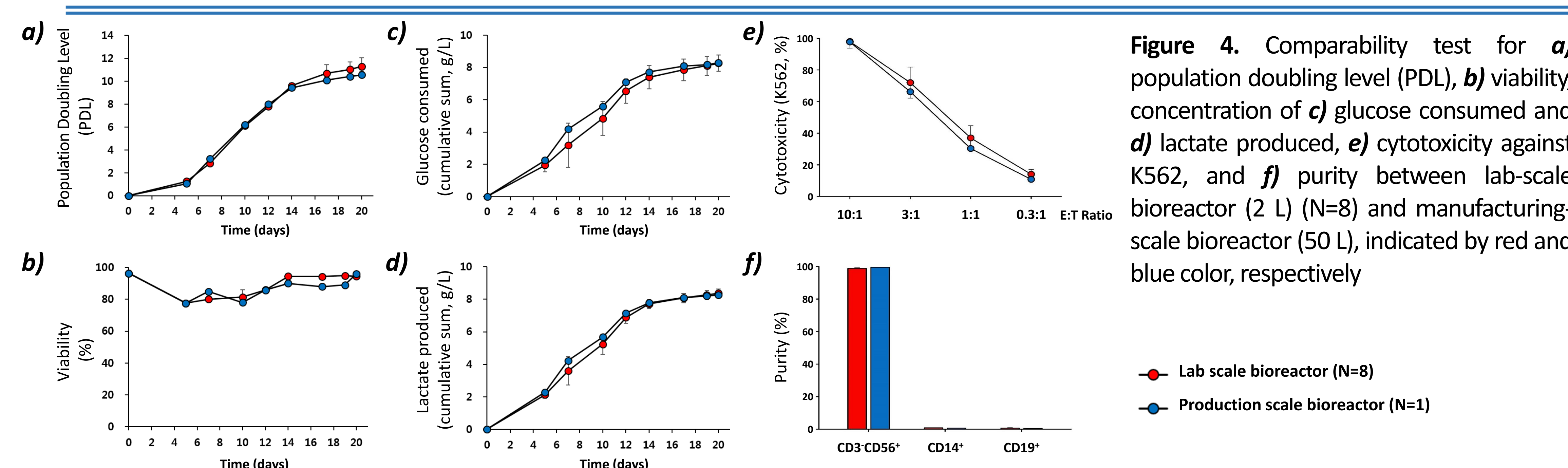
PERFORMANCE COMPARISON OF SCALES

Figure 4. Comparability test for *a)* population doubling level (PDL), *b)* viability, concentration of *c)* glucose consumed and *d)* lactate produced, *e)* cytotoxicity against K562, and *f)* purity between lab-scale bioreactor (2 L) (N=8) and manufacturing-scale bioreactor (50 L), indicated by red and blue color, respectively

Lab scale bioreactor (N=8) — red
Production scale bioreactor (N=1) — blue

Population doubling level (PDL), viability, the cumulative concentration of glucose consumed and lactate produced were measured with time. All the IPC data at 50-L scale showed to be comparable with 2 L scale. Harvested NK cells from 50 L bioreactor were analyzed for cytotoxicity and purity. It was confirmed that manufacturing scale cultivation was also comparable with its cytotoxicity against K562 cells and purity.

CONCLUSION

Bioreactor process at 50 L scale was demonstrated to be comparable as at 2 L scale in producing allogeneic NK cells. Scale-up parameters were optimized with classical engineering parameters and advanced engineering parameters. The similarity of classical parameters (power input (P/V), Newton number (Ne), geometry) helped to calculate which RPM could be operated at 50 L scale. In addition to that, Infinitesimal analysis based on advanced parameters, showed that 50 L scale operation with the RPM lowered shear stress but increased turbulent kinetic energy. Under the circumstance, PDL (~12), viability (>95%), cytotoxicity, and purity (>99% of CD3-CD56+) of cells at 50 L scale were almost comparable with ones at 2 L scale. The developed process will be applied to produce clinical materials for phase III.

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