**Supplementary Material:**

Table 1: Statistical analysis of T-cells subpopulations subjected to different feeding dilution frequencies and initial seeding density scenarios in Monod Kinetics model1. Cells with starting density of 1 x 106 cells/ mL or 0.5 x 106 cell/ mL were subjected to one dilution feed at either 28hr or 40hr post inoculation in Ambr®15 vessels over 5 days culture. CD8+ TEM population exhibited more sensitivity to the change over time in culture, feeding frequency and initial seeding density but overall, starting seeding density showed a dominant effect on both CD4+ and CD8+ sup populations including; CD4+ TEM, CD4+ effector and CD8+ SCM/ TCM and CD8+ effector and CD4+,CD8+ percentage.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| % Dilution | Day | CD4 naïve | CD4 SCM/TCM | CD4 TEM | CD4 effector | CD8 naïve | CD8 SCM/TCM | CD8 TEM | CD8 effector | CD4% | CD8% |
|  | 0 | 0.98 | 13.4 | 80.15 | 5.46 | 0.645 | 6.13 | 85.6 | 7.63 | 57 | 19.15 |
| 28h HD | 2 | 6.35 | 52.55 | 35.65 | 4.33 | 12.35 | 63.5 | 19.45 | 4.2 | 47 | 28.25 |
| 40h HD | 2 | 6.26 | 45.95 | 42.55 | 5.74 | 11.05 | 50.6 | 29.7 | 9.62 | 45.95 | 23.25 |
| 28h LD | 2 | 7.305 | 41.05 | 46.5 | 7.135 | 12.5 | 45.7 | 31.1 | 13.1 | 45.15 | 26.3 |
| 40h LD | 2 | 7.305 | 41.05 | 46.5 | 7.135 | 15.35 | 47.9 | 26.9 | 9.385 | 46.7 | 25.75 |
| 28h HD | 5 | 10.115 | 55.6 | 25.15 | 9.12 | 14.1 | 25.45 | 36.7 | 23.8 | 63.35 | 17.05 |
| 40h HD | 5 | 9.975 | 53.1 | 25.15 | 11.785 | 20 | 28.35 | 32.25 | 19.45 | 59.7 | 12.6 |
| 28h LD | 5 | 3.9 | 30.25 | 51.85 | 13.95 | 17.25 | 19.45 | 34 | 29.3 | 43.6 | 27.3 |
| 40h LD | 5 | 5.775 | 27.85 | 48.05 | 20.3 | 18.05 | 15.1 | 31.85 | 34.45 | 47.45 | 31.4 |
|  |  |  |  |  |  |  |  |  |  |  |  |
| DayP value |  | 0.928 | 0.230 | 0.346 | 0.000 | 0.001 | 0.001 | 0.016 | 0.000 | 0.021 | 0.187 |
| Dilution timeP value |  | 0.481 | 0.436 | 0.803 | 0.075 | 0.081 | 0.041 | 0.996 | 0.941 | 0.950 | 0.597 |
| Density P value |  | 0.239 | 0.000 | 0.002 | 0.005 | 0.216 | 0.003 | 0.545 | 0.001 | 0.011 | 0.018 |

Table 2: Statistical analysis of T-cells subpopulations subjected to a variety of feeding dilution scenarios in glucose supply experiment model 2. Feeding dilutions of 1 %, 1.13 % and 0.57 % medium were added hourly to T-cell suspension culture. The percentage feed started 18 hr post inoculation in Ambr®15 vessels and maintained over 6 days of experiment. Changing time in culture seemed to have a more dominant influence on percentage of CD4+ subpopulations and CD8+ TEM in comparison with different dilution conditions.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| % Dilution | Day | CD4 naïve | CD4 SCM/TCM | CD4 TEM | CD4 effector | CD8 naïve | CD8 SCM/TCM | CD8 TEM | CD8 effector | CD4% | CD8% |
|  | 0 | 2.76 | 64.1 | 32.6 | 0.54 | 3.16 | 43.9 | 47.1 | 5.825 | 51.95 | 20.6 |
| 1.00 | 3 | 0.69 | 65.75 | 33.4 | 0.1605 | 2.275 | 55.3 | 40.8 | 1.64 | 62.75 | 20.7 |
| 1.13 | 3 | 0.72 | 67.15 | 31.95 | 0.195 | 2.615 | 57.25 | 38.45 | 1.66 | 62.7 | 18.45 |
| 0.57 | 3 | 0.655 | 63.5 | 35.55 | 0.265 | 3.02 | 50.3 | 44.7 | 2.035 | 60.65 | 21.95 |
| 1.00 | 6 | 1.15 | 54.55 | 42.85 | 1.405 | 3.07 | 49.4 | 46.85 | 0.69 | 57.6 | 15.8 |
| 1.13 | 6 | 3.465 | 52 | 44.5 | 0.745 | 1.335 | 66.65 | 31 | 1.27 | 62.6 | 15 |
| 0.57 | 6 | 3.55 | 50.1 | 45.8 | 1.155 | 2.94 | 57.7 | 40.15 | 1.375 | 50.55 | 16.6 |
|  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Day P value |  | 0.001 | 0.000 | 0.000 | 0.001 | 0.711 | 0.257 | 0.471 | 0.005 | 0.049 | 0.001 |
| DilutionP value |  | 0.079 | 0.215 | 0.377 | 0.079 | 0.286 | 0.064 | 0.045 | 0.09 | 0.080 | 0.093 |

Table 3: Statistical analysis of T cells subpopulations subjected to a variety of feeding dilution scenarios in optimised glucose supply experiment model 3. Feeding dilution of 0.6 %, 0.8 % and 1 %, and glucose supply conditions including; 1 % feed with 20 % and 40 % added glucose that were added hourly to T cell suspension culture. The percentage feed started 18 h post inoculation in Ambr®15 vessels and maintained over 7 days of experiment. Time in culture had a greater influence on selection of T cell subsets and sub populations in comparison with different dilution and glucose supply scenarios. CD8+ populations ofnaïve, TEM, effector and CD8+ percentage changed significantly over time in either dilution feed or glucose supply scenarios. Between all experimental groups, CD8+ SCM/TCM population manifested a substantial sensitivity to changes in both dilution and glucose supply conditions (P value ≤ 0.05).

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| dilution% | day | CD4 naïve | CD4 SCM/TCM | CD4 TEM | CD4 effector | CD8 naïve | CD8 SCM/TCM | CD8 TEM | CD8 effector | CD4% | CD8% |
|  | 0 | 9.16 | 42 | 48.4 | 1.765 | 10.65 | 6.16 | 67.7 | 15.9 | 44 | 14.80 |
| 1.00 (0) | 4 | 1.14 | 68 | 29.25 | 1.62 | 4.075 | 56.25 | 39 | 0.7 | 50.1 | 22.75 |
| 1.00 (20%) | 4 | 1.915 | 69.75 | 27.45 | 1.105 | 6.015 | 56.85 | 37.65 | 0.865 | 51 | 22.85 |
| 1.00 (40%) | 4 | 1.975 | 67.15 | 28.96 | 1.835 | 5.44 | 55.45 | 37.15 | 1.1 | 47.7 | 21.45 |
| 1.00 (0) | 7 | 5.855 | 71.615 | 16.95 | 3.36 | 2.86 | 40.35 | 52.7 | 2.96 | 72.85 | 2.93 |
| 1.00 (20%) | 7 | 2.285 | 86.1 | 12.85 | 1.475 | 1.67 | 42.2 | 53.55 | 7.09 | 78.65 | 6.32 |
| 1.00 (40%) | 7 | 0.935 | 70.7 | 20.35 | 1.26 | 5.095 | 31.7 | 63.75 | 1.86 | 67.6 | 15.9 |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Day P value |  | 0.260 | 0.069 | 0.000 | 0.311 | 0.024 | 0.000 | 0.000 | 0.012 | 0.000 | 0.001  |
| Glucose increaseP value |  | 0.357 | 0.157 | 0.077 | 0.155 | 0.155 | 0.010 | 0.116 | 0.126 | 0.269 | 0.274 |
| dilution% | day | CD4 naïve | CD4 SCM/TCM | CD4 TEM | CD4 effector | CD8 naïve | CD8 SCM/TCM | CD8 TEM | CD8 effector | CD4% | CD8% |
|  | 0 | 9.16 | 42 | 48.4 | 1.765 | 10.65 | 6.16 | 67.7 | 15.9 | 44 | 14.80 |
| 0.6% | 4 | 1.26 | 63.35 | 33.4 | 2.005 | 3.05 | 46.45 | 48.4 | 0.785 | 49.3 | 22.9 |
| 0.8% | 4 | 1.5 | 65.85 | 32.05 | 0.85 | 3.785 | 49.45 | 45.95 | 0.8 | 52.45 | 22.35 |
| 1.00  | 4 | 1.14 | 68 | 29.25 | 1.62 | 4.075 | 56.25 | 39 | 0.7 | 50.1 | 22.75 |
| 0.6% | 7 | 3.57 | 76.9 | 19.3 | 1.24 | 5.16 | 35.75 | 56.25 | 2.1 | 78.8 | 5.7 |
| 0.8% | 7 | 0.835 | 75.85 | 21.15 | 0.885 | 4.695 | 20.75 | 71.85 | 3.475 | 74.8 | 74.8 |
| 1.00  | 7 | 5.855 | 71.615 | 16.95 | 3.36 | 2.86 | 40.35 | 52.7 | 2.96 | 72.85 | 9.005 |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Day P value |  | 0.077 | 0.005 | 0.000 | 0.318 | 0.384 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000  |
| DilutionP value |  | 0.248 | 0.899 | 0.063 | 0.023 | 0.607 | 0.020 | 0.019 | 0.492 | 0.819 | 0.604 |

Table 4: Multi-level Model to predict process outcome

|  |  |  |  |
| --- | --- | --- | --- |
|  | **First Level Model**  **(FLM)** | **Second Level Model**  **(SLM)** | **Third Level Model (TLM)** |
| Purpose | ID approximate operational constrainsID development control issuesID what needs engineering | Increased definition of challenging operations/sub-processes/mechanisms | Define manufacturing tolerancesDefine operating space and predict risk |
| Required input | Low knowledgeLow data | FLM knowledge refining data | FLM/SLM knowledge refining data |
| Characteristics | Conserved behavioursAggregated mechanismsKey operation relevant | System specific behaviours | All manufacturing critical system behaviours |

 

Figure 1: Examples of manual gating of flow data. A and B are representatives of gating for CCR7 and CD45RA antibodies in CD4+ and CD8+ positive T-cells in experimental condition with 1 % dilution feed and 20 % additional glucose into bulk medium at day 4.