Seeding Distribution in Channel of the Cell Culture Vessel

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Abstract: Conventional cell culture (CC) techniques are advancing significantly with a trend towards a mass production. While plating density (number of cells per volume of culture medium) plays a critical role for some cell types, the initial distribution of these cells is affected by the actual size and shape of the space in which cells are introduced. This work focuses on the analysis of cell distribution during seeding into a serpentine channel of CC vessel.

Keywords: cell culture, plating density, seeding distribution.

1. Introduction

Conventional cell culture (CC) techniques are advancing significantly with a trend towards a mass production. For example, the induced pluripotent cells (iPSC) strategy and studies are aimed to gain insight into how to reprogram cells to repair damaged tissues in the human body. The most commonly varied factor in a culture system is the cell growth medium. Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrients. Plating density (number of cells per volume of culture medium) plays a critical role for some cell types. The suspension and cell transport at various process steps will have some impact on cells' position inside growth zone. During uploading and growth process it is important to provide and to sustain *in-situ* and *after seeding* distribution of the cells inside macro-channels of the CC vessel. As cells generally continue to divide in culture, they generally grow to fill the available area or space. The initial distribution will depend on the actual size and shape of the space in which cells are introduced, uploading procedure into seeding positions, impact of the nutrition media exchange, etc. This work focuses on the analysis of cell distribution during seeding into the channel of CC vessel designated for mass-production of the cells.

2. Model and Methods

To simulate the plating density distribution we proceeded with considering a laminar incompressible fluid flow model. Due to a small Reynolds number in a low-volume flow we neglected any turbulence inside the channel. When a steady flow is established, a dose of cells is introduced into fluid prior to releasing into the channel. Since the mass density of the suspension liquid and of cells is very close to each other in values, 1 g/cm^3 and 1.095 g/cm^3, respectively, the effect of drag force is expected to be high while cell sedimentation time is expected to be long. Moreover, buoyancy may balance gravity during flow. Because of the limited time steps ($t_{seeding} < t_{sediment}$) cell sedimentation during the modeling timeframe is expected to be negligible, though it was coupled into model. Process temperature was set to 35 °C. Cells were considered to have the a shape of spheres with diameter 15 **μ**m.

2.1. Use of COMSOL Multiphysics

In this work we used physics interfaces *Laminar Flow* and *Particle Tracing for Fluid Flow* that solves interaction under COMSOL Multiphysics (v.4.4). Two studies were formulated in a model tree: (1) stationary *Study 1* for laminar incompressible fluid flow and (2) transient *Study 2* on particle tracing. Inflow

velocity of the suspension was estimated from volumetric flow rate. Initial conditions in computational domain were set to $u_0 = 0$ and $p_0 = 0$. The *Study 1* was solved very quickly in less than 30 min.

The second *Study 2* – a transient computation of cells pathways was initially set to the *range(0,0.5,300)*. As we found out later that it was sufficient to compute only 120 s (instant pulse of the cells traveled through the channel in times shorter than 60 s). Here and through the text below, the term *channel* refers to a straight section of the vessel. There are 23 channels in a CC vessel. The computation of the particles tracing in seeding step within 120 s for instant dose that contains 10k cells took about 35 hours on PC with 24 cores. To have available data on particle tracing it is important to check box *Store Particle Status Data* in *Advanced Settings* of the interface *Particle Tracing for Fluid Flow*.

2.2 Computational Domain and Conditions

The transit domain of the CC vessel was formed with a serpentine type fluidic design having 23 channels with a cross-section area of approximately 12 mm^2 and total length about 1.4 m (Fig. 1). We assumed free flow – that is, the "air-plug" was not considered in this simulation. *No slip* conditions were set on walls in contact with the suspension fluid. Particle were reflected when hitting walls (*bounce* conditions) except the outlet surface (*disappear* conditions). In the model cell releasing either in a single pulse mode or in a continuous mode was formulated. Instant release contained 10k cells at t=0.

Continuous dose at the needle inlet (area 1.12 mm^2) was assumed ~1.65×10^9 cells/(m^{2}s). Within the initial 60 s solver should trace over 222,000 cells inside vessel which might slow down computation. As we noted, during computations the number of cells introduced into vessel and used in models should be at least or above 30 % value of the real process dose to minimize statistical (cumulative) error, however, the computation time increases substantially as the number of cells introduced into computational domain increases. The computer (2x12 cores machine) utilized almost 24 hours of machine time to compute duration of 8.5 s within transient model Obviously, to achieve meaningful output data for analysis it is

necessary to run large enough number of the cells through the CC vessel.
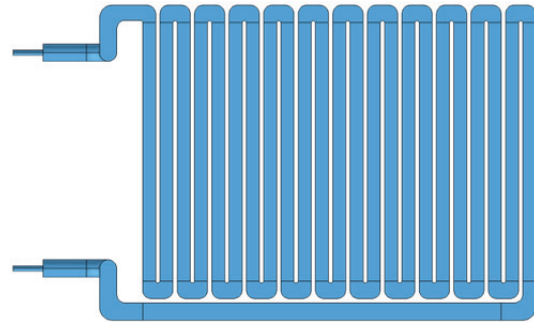
Figure 1. The serpentine type fluidic channel of the cell culture vessel.
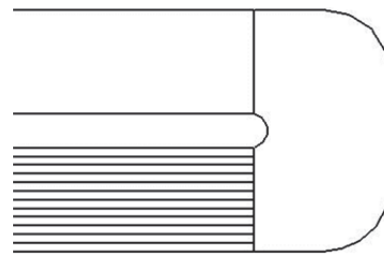
Figure 2. The first, middle (12) and 21st channels of the cell culture vessel were split into multiple bins (12) to track and analyze cumulative distribution across the flow.

To quantify cell distribution, selected channels were split into several bins (Fig. 2) in the direction normal to the suspension flow. Post-processing of the computed data extensively utilized the particle tracing capabilities *Export Animation* and *Export Data*. For instance, the variable *Total number of particles in selection (fpt.Nsel)* was very useful and practical in settings of our analysis. The actual distributions of the cells were analyzed either in *Excel* or by *Table Graph* tool in COMSOL.

2.3 Governing Equations

The incompressible fluid flow in the channel is governed by Navier-Stokes equations (Eqs. 1 and 2).

$$\rho \frac{\partial \boldsymbol{u}}{\partial t} - \nabla \cdot \eta (\nabla \boldsymbol{u} + (\nabla \boldsymbol{u})^T) + \rho \boldsymbol{u} \cdot \nabla \boldsymbol{u} + \nabla p = 0 \quad (1)$$

$$\nabla \cdot \boldsymbol{u} = 0 \quad\quad\quad\quad\quad\quad\quad (2)$$

During the seeding process, cells are exposed to a drag force, buoyancy and gravitation force and their position is tracked by Eq. 3.

$$\frac{d(m_{cell} \boldsymbol{v}_{cell})}{dt} = \boldsymbol{F}_d + \boldsymbol{F}_g + \boldsymbol{F}_b \quad\quad (3)$$

where m_{cell}, v_{cell}, F_d, F_g and F_b is cell mass, velocity, drag force, gravitation and buoyancy.

## 3. Results

3.1 Simulation

Stationary study on fluid flow transport yielded the highest estimate for the velocity of the liquid media, up to 4 cm/s. The velocity field is uniformly formed through all channels. Cells introduced into the vessel are immediately dragged by flowing suspension and the spreading of the cells downstream depends on their initial {y,z} coordinates at the inlet and geometry of the inlet section (Fig. 3).
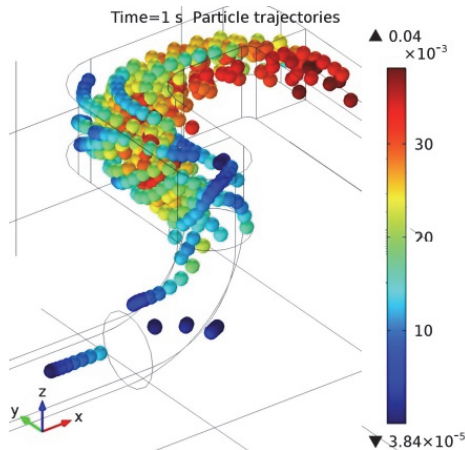

Figure 3. A single release of the 10k cells is illustrates immediately spread within inlet section. Cell size is scaled 10x, the color corresponds to velocity of the cells.

In the case of the single instant release of the 10k cells the cumulative count of the cells in

individual channels is given in Fig. 4. The initial width of the cells envelope, $\Delta t \rightarrow 0$, gradually increases almost up to $\Delta t \approx 20$ s when reaching outlet section. This is illustrated in Fig. 5 at different instance as cells' envelope propagates through the vessel.
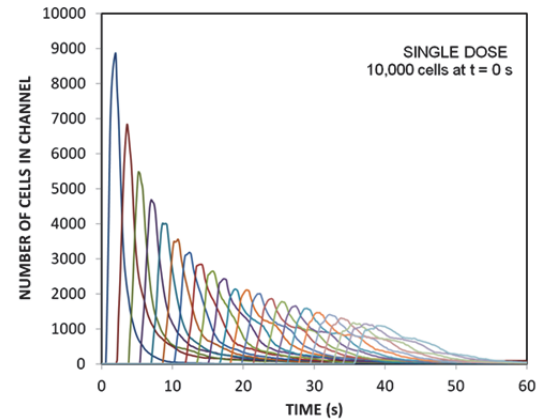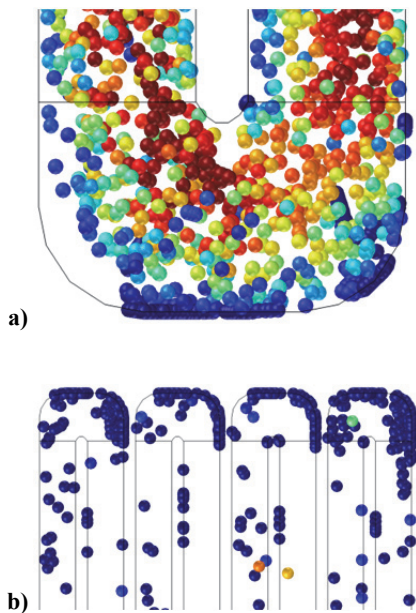

Figure 4. Cumulative cell count in individual channels during a single release of the 10k cells as cells envelope propagates through the vessel.

a)

b)

c)

d)
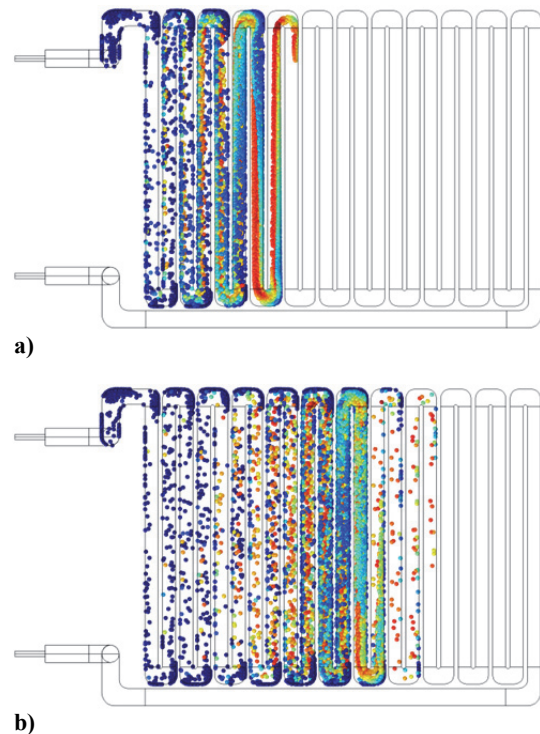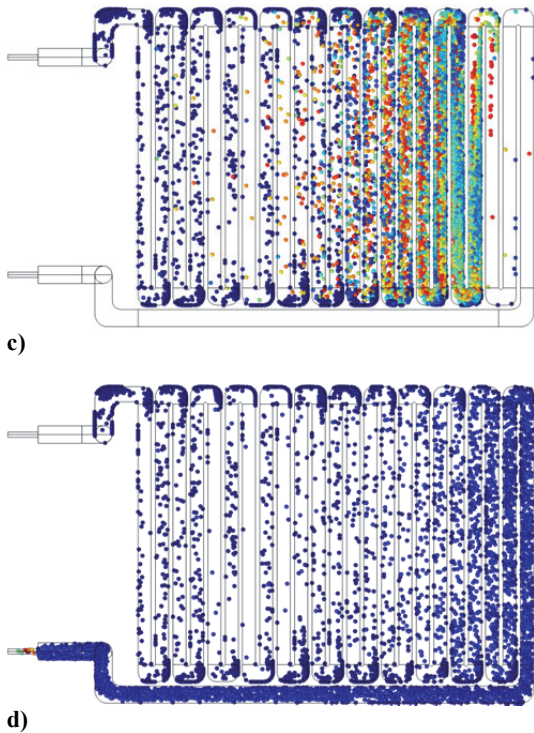
Figure 5. Transition of cells' envelope through vessel in (a) 15 s, (b) 30 s, (c) 45 s and (d) 60 s. Cell size is scaled 10x, the color corresponds to velocity of cells.


a)

b)

Figure 6. Slowed down cells are observed close to walls and in U-loops (a). Image (b) shows remaining

cells inside channel or in U-loops even after time t=120 s, when the initial cell envelope passed completely through the outlet. Cell size is scaled 10x, the color corresponds to velocity of cells.

Images (a) and (b) in Fig. 6 show cells in U-turn sections during and after seeding flow, respectively. Noticeably an increase in cell accumulation is seen at low velocity (Fig. 6-a, see also Fig. 5-d) during transport of cells. Even after cells envelope left the vessel ($t > 100$ s) some cells remain inside the channels (Fig. 6-b).

Residual cells play two-fold role: increasing plating density upstream but depleting downstream seeding density. On the other side, the plating density produced by single pulse seeding is depleted twice the number as the cell envelope moves through the vessel, however, the cells residence time in channels is increasing from 2 s to 7 s (channel 1 vs 21st channel).

From the single (instant) release simulation we determined that the minimal transit time of cells through the vessel is about 55-60 sec and maximum is up to ~ 100 sec. Reduction of such spread may be achieved by introducing an air-plug into seeding sequence. The air-plug will maintain front velocity at constant level thus uniform plating density downstream the channels, thus uniform sedimentation of the cells on the bottom of the channel.
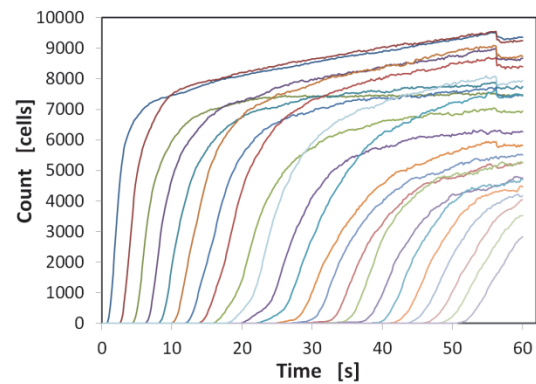
Figure 7. Cumulative cell count during a continuous dose at the inlet of CC vessel. Curves represent cell number in individual channels.

Regarding the question *"what is the in-situ and after seeding distribution of the cells inside a CC*

vessel when using a releasing mode of continuous dose?" The answer can be obtained from downstream distribution of the cells in the model of continuous release of the cells. Figure 7 shows the cumulative cell count during a continuous dose at the inlet of the CC vessel. The curves represent cumulative cell number in individual channels. Initial increase in cell count is due to new cells entering into particular channel during seeding step. The quasi saturation is formed due to outgoing cells from channel. Slight increase during saturation phase in several bins is due to stacked residual cells. Images (a) and (b) in Fig. 8 show cells transition through a vessel in continuous release mode and completing seeding process in 60 s.


a)

b)
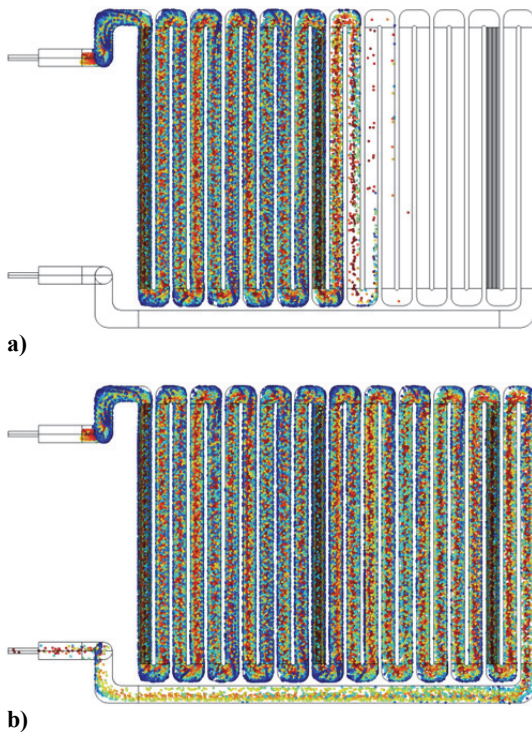
Figure 8. Transition of cells in a mode of continuous dose in (a) 30 s and (b) 60 s. Cell size is scaled 10x, the color corresponds to velocity of cells.

The channel distribution in normal direction is estimated from cumulative densities in the individual bins. Figure 9 shows cross-channel *"after-sedimentation"* distributions in bins of channel 1, 12 and 21.
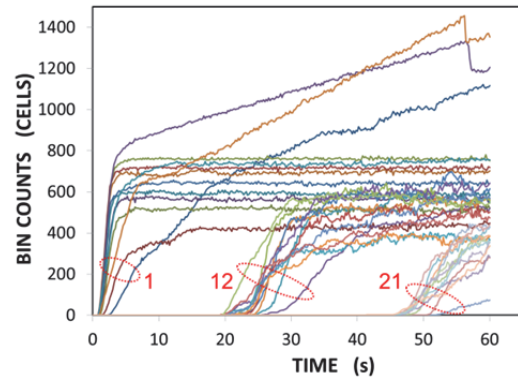
Figure 9. Cumulative cell counts per bin in channel 1, 11 and 21. Continuous dose at the inlet of CC vessel. Curves represent cell number in individual channels (normalized data).
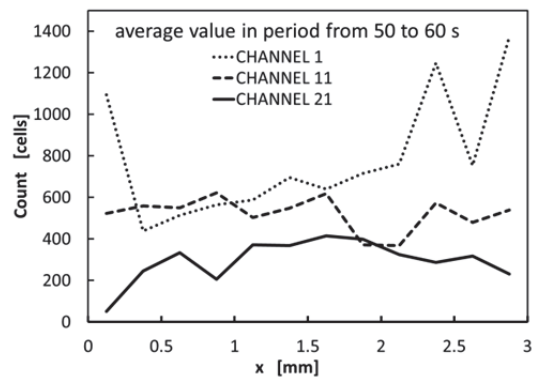

Figure 10. Cross-channel *"after-sedimentation"* (cumulative) distributions in channel 1, 12 and 21.

Channels closer to the inlet exhibited edge heavy plating density, channels closer to the outlet exhibited distribution that correspond more to velocity profile. This trend can be explained by reduced velocity at sidewalls due to boundary conditions. That means the propagating cell population is depleted at sidewalls during the transport and positions distanced far more from inlet are seeded less at sidewall in respect to center. Average distribution inside the 1st, 12th and 21st channel at time within interval 50-60 s is illustrated in Fig. 10. Thus, downstream the channels the distribution is getting more uniform. Further

improvement of uniformity is established by air-plug mode.

3.2 Experiments and Discussion

The simulation results discussed above were compared with experimental observations. It can be anticipated that resulting plating density at the bottom surface of the channel will be integral image of the volumetric plating density at the instant when flow is stopped and sedimentation takes place.

a)   b)

c)   d)
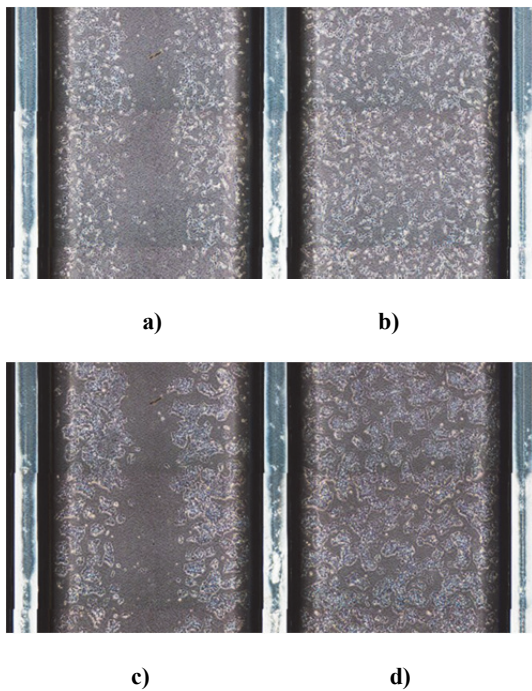
Figure 11. Experimental distribution of the cells in straight sections: (a) close to inlet and (b) is more distanced from inlet and observed within 24 hours after seeding. Images (c) and (d) show same locations after 3 days growth.

The experimental results were assessed in various locations through the CC vessel and compared with modeling outputs. We used stated interpretation above, though, we are aware that in reality the overall seeding process could be much more complex. Nevertheless, the agreement between experimental and simulation distributions was observed. Figure 11 shows the cells distributions inside channel. Increased towards the walls plating density within 24 hours after seeding is noticeable in positions that are relatively closer to the inlet Fig. 11-a. Far away from inlet the distribution is more uniform (Fig. 11-b). Images (c) and (d) in Figure 11 show same locations as (a) and (b) after 3 days growths.

Image in Figure 12 shows slightly higher plating density distribution in one of many U-turns. This result can be well correlated with simulation output shown above in Fig. 6-a.

Figure 12. Experimental distribution of the cells in curved sections.
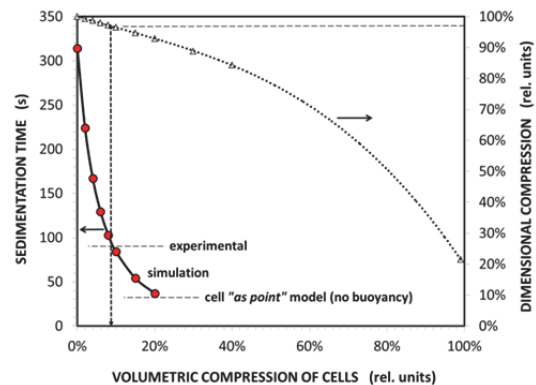
Figure 13. Simulation of the sedimentation time at various compression of cell at constant cell mass.

Due to statistical nature in size ($\varnothing 14.7 \pm 2.0$ **µ**m) and position of the cells inside vessel prior sedimentation one can estimate only average sedimentation time. From experimental data it was determined that sedimentation occurs at rate

about 1 mm per 90 s. If considering cell as a virtual point (radius → 0) there is no buoyancy force on cell and sedimentation will not occur in model. Considering finite cell radius and possible cell compression inside liquid the simulation (Fig. 13) provided good match in sedimentation time at about 97 % dimensional compression of the cells that is leading to small increase in mass density of the cell.

4. Conclusions

Simulation of the cell seeding distribution showed increased plating density due to boundary layer conditions in off-centerline and loop locations. Transit and sedimentation times estimated by simulation correlate well with experiment. Slight volumetric compression of cells ~9% was determined by matching a buoyancy force to experimental sedimentation time. Simulation indicated possibility towards higher yield performance of the cell culture vessel.