Supplementary Data

This MATLAB software with a simple user interface is provided as supplementary material, enabling researchers to simply enter the parameters of their system (e.g., concentration of substance, metabolic rates of cells, and size of 3D construct) and model the diffusion gradient in the tissue construct according to the equations presented herein.

Instructions for Supplementary MATLAB Script

1. Save the “Interface.m” and “Interface.fig” files to the same location, and then run the “Interface.m” file in MATLAB. A user interface window will then pop up as shown in the screenshot image, in which the settings and parameters of the tissue system can be entered and adjusted, and in which the final graph showing diffusion solutions will be displayed.

2. In the user interface window, choose the shape that best represents the tissue construct and diffusion system.
   - For flat sheets or slab-like constructs, diffusion is primarily unidirectional: Choose 1D.
   - For cylindrical constructs, diffusion primarily occurs through a round interface: Choose 2D.
   - For spherical constructs, diffusion occurs through all directions: Choose 3D.

3. Choose the conditions that best represent the system.
   - Choose whether the diffusant molecule is diffusing into or out of the tissue construct.
   - Choose whether the diffusant molecule is metabolized (like many nutrients) or not metabolized (like many signaling factors).

4. Enter the parameters of the system.
   - Enter the initial concentration of diffusant molecule at its source at the interface of the tissue construct (in mM, e.g., 10 mM glucose).
   - Enter the desired duration of time for analysis (in seconds, e.g., 10,000 s).
   - Enter the diffusion coefficient for the type of tissue construct (in mm²/s).
   - Enter the average metabolic rate of consumption (φ) of the diffusant molecule per cell (in mol/Ls).
     - Note: generally in hydrogels the diffusion coefficient is around 10⁻³ for oxygen, 10⁻⁴ mm²/s for glucose, and 10⁻⁵ mm²/s for proteins [4].
     - Enter the thickness of the tissue slab or the radius of the round tissue construct (in mm).
   - Enter the total volume of the tissue construct (or simply allow the computer to calculate this value from the prior entry by assuming that the construct is a cube or a sphere) (in mm³, e.g., 4 mm³).
   - Enter the volume of media in which the tissue construct is cultured (in mm³, e.g., 5000 mm³).

5. Click the “Run” box in the user interface, and after a bit of processing, a 3D graph of results will be produced.
   - The spatial axis (to the right) represents the thickness or radial depth of the tissue construct.
   - The time axis (to the left) represents the length of time after initial starting conditions.
   - The vertical axis represents the concentration of the diffusant molecule over time and space.
   - The cursor tool at the top of the graph may be used to find exact values at any point in space and time.

Additional Notes for the User

1. It should be noted that the metabolic rate of the tissue construct (φ) equals the average metabolic rate of the cell (m) multiplied by the average density of the cells in the tissue construct (ρ). It should also be noted that the theoretical maximum thickness or maximum radius of a tissue construct is determined by the metabolic rate of consumption of the diffusant (φ):

   \[ T_{max} \text{ or } R_{max} = \sqrt{\frac{2mC_oD}{\phi}} \]

   Thus, to avoid confusion or user error in the MATLAB program, only the maximal thickness or radius of the
tissue construct can be entered in the user interface (rather than the metabolic rate per cell) since one inherently leads to the other, and since the maximal tissue construct size can also simply be found empirically as well as by calculation.

2. For those without access to MATLAB, other open source software languages would also be capable of implementing these models (eg, Julia, Python, Sage, Octave, Scilab).

**Supplementary Table S1. Diffusion Model Boundary Conditions**

| Eq. 1 | \( C(x, 0) = C_o \) for \( 0 \leq x \leq T \) |
|---|---|
| \( C(0, t) = 0 \) for \( t > 0 \) |
| \( \partial C(\infty, t)/\partial x = 0 \) |

| Eq. 2 | \( C(r, 0) = C_o \) for \( 0 \leq r \leq R \) |
|---|---|
| \( C(R, t) = 0 \) for \( t > 0 \) |
| \( \partial C(0, t)/\partial r = 0 \) |

| Eq. 3 | \( C(r, 0) = C_o \) for \( 0 \leq r \leq R \) |
|---|---|
| \( C(0, t) = 0 \) for \( t > 0 \) |
| \( \lim_{r \to 0} C(r, t) = \text{bounded} \) |

| Eq. 4 | \( C(x, 0) = 0 \) for \( 0 \leq x \leq T \) |
|---|---|
| \( C(0, t) = C_o \) for \( t > 0 \) |
| \( \partial C(\infty, t)/\partial x = 0 \) |

| Eq. 5 | \( C(r, 0) = 0 \) for \( 0 \leq r \leq R \) |
|---|---|
| \( C(R, t) = C_o \) for \( t > 0 \) |
| \( \partial C(0, t)/\partial r = 0 \) |

| Eq. 6 | \( C(r, 0) = 0 \) for \( 0 \leq r \leq R \) |
|---|---|
| \( C(R, t) = C_o \) for \( t > 0 \) |
| \( \lim_{r \to 0} C(r, t) = \text{bounded} \) |

| Eq. 7 | \( C(x, 0) = 0 \) for \( 0 \leq x \leq T \) |
|---|---|
| \( C(T, t) = 0 \) |
| \( C(0, t) = C_o \) |

| Eq. 8 | \( C(r, 0) = 0 \) for \( 0 \leq r \leq R \) |
|---|---|
| \( C(R, t) = C_o \) |
| \( C(0, t) = 0 \) |

| Eq. 9 | \( C(r, 0) = 0 \) for \( 0 \leq r \leq R \) |
|---|---|
| \( C(R, t) = C_o \) |
| \( C(0, t) = 0 \) |

| Eq. 10 | \( C(x, 0) = 0 \) for \( 0 \leq x \leq T \) |
|---|---|
| \( C(T, t) = 0 \) |
| \( C(0, t) = C_o - \frac{\rho U t V_r}{V_m - V_r} \) |
| \( C(\infty, t) - \frac{V_r}{V_m - V_r} \) |

| Eq. 11 | \( C(r, 0) = 0 \) for \( 0 \leq r \leq R \) |
|---|---|
| \( C(R, t) = C_o - \frac{\rho U t V_r}{V_m - V_r} \) |
| \( C(0, t) = 0 \) |

| Eq. 12 | \( C(r, 0) = 0 \) for \( 0 \leq r \leq R \) |
|---|---|
| \( C(R, t) = C_o - \frac{\rho U t V_r}{V_m - V_r} \) |
| \( C(0, t) = 0 \) |

The initial and boundary conditions of each respective equation are provided.