

Modeling of an Oxygenation-Aided 3D Culture for Functional Beta-Cell Expansion

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Introduction: One promising method for the treatment of diabetes is the transplant of insulin-producing pancreatic beta cells in order to increase beta cell mass and improve the patient's ability to regulate their own blood-glucose levels. Currently, researchers are looking for ways to mass-produce biologically functional beta cells *in vitro* because of the shortage of donor tissue. Unfortunately, beta cells proliferate slowly, often lose function, and require large amounts of oxygen *in vitro*. The beta cells will become hypoxic if these oxygen demands are not met. Beta cell viability can be improved through the use of O₂-producing materials. We have found that beta cells that are cultured in a 3D collagen scaffold secrete more insulin than beta cells cultured in 2D conditions. We hypothesized that the biological function of beta cells can be improved if they are cultured in a 3D collagen scaffold, which mimics physiologic conditions *in vivo* and by using an oxygen-producing biomaterial to meet oxygen demands. To test this hypothesis we used COMSOL Multiphysics software to simulate and optimize the oxygenation of our culture environment.

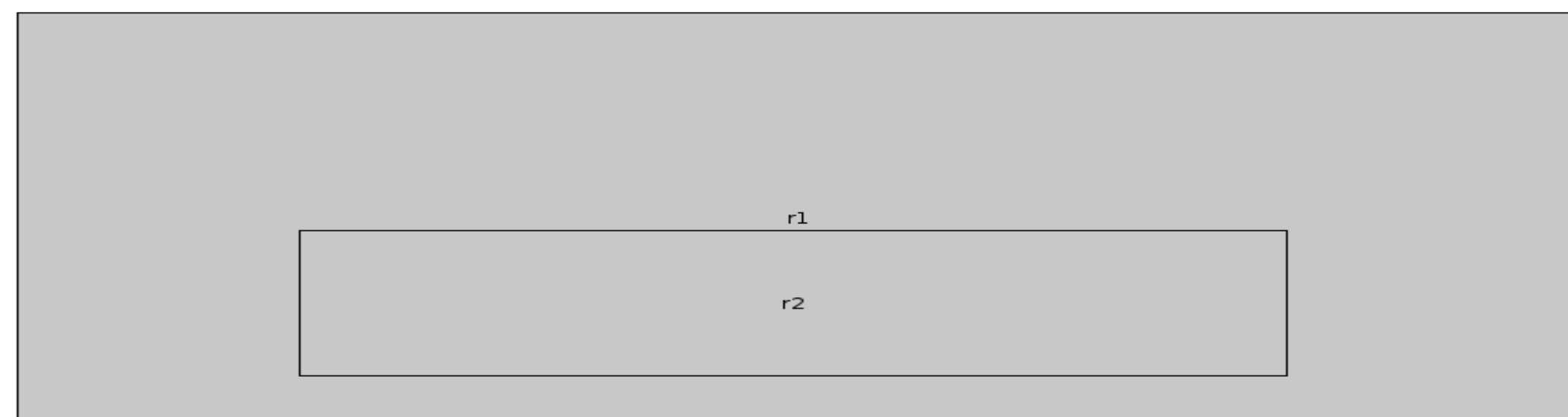


Figure 1. The basic geometry of the culture system, where a 3D scaffold (d=11.05mm, h=4.25 mm) is placed in one well of a 48-well cell culture plate with a PDMS-H₂O₂ disk (d=7mm, h=1.5mm)

Computational Methods: Our model was designed to determine the ability of our O₂-producing material to sufficiently oxygenate a beta cell culture. Figure 1 shows the basic geometry of our model. The Transport of Diluted Species physics setting was used. The culture medium was assumed to consist of water with a collagen concentration of 1.5 mg/ml (oxygen diffusion coefficient of 2.99e-9 m²/s). The beta cells were assumed to be uniformly distributed throughout the culture media, and to consume oxygen by Michaelis-Menten kinetics ($V_{max} = 6.367e-17$ mol/(s*cell), and $K_m = .005$ mol/m³). The oxygenator was made of H₂O₂ encapsulated within a PDMS disk. The PDMS disk is permeable to oxygen (diffusion coefficient 6e-9 m²/s), and water (diffusion coefficient 2.49e-9 m²/s). After measuring the oxygen release rate of the oxygenators over a period of two weeks, it was determined that the oxygenator released oxygen based on Michaelis-Menten kinetics with $V_{max} = 2.572e-6$ mol/(m³*s), and $K_m = 5.34e-5$ mol/m³. The top boundary of the culture was assumed to be open to the air, which was assumed to have an oxygen concentration of 0.2 mol/m³.

Results: To determine whether or not the oxygenator (PDMS-H₂O₂ disk) could effectively be used to improve and optimize oxygenation of the culture various simulations were performed, as shown by the following figures.

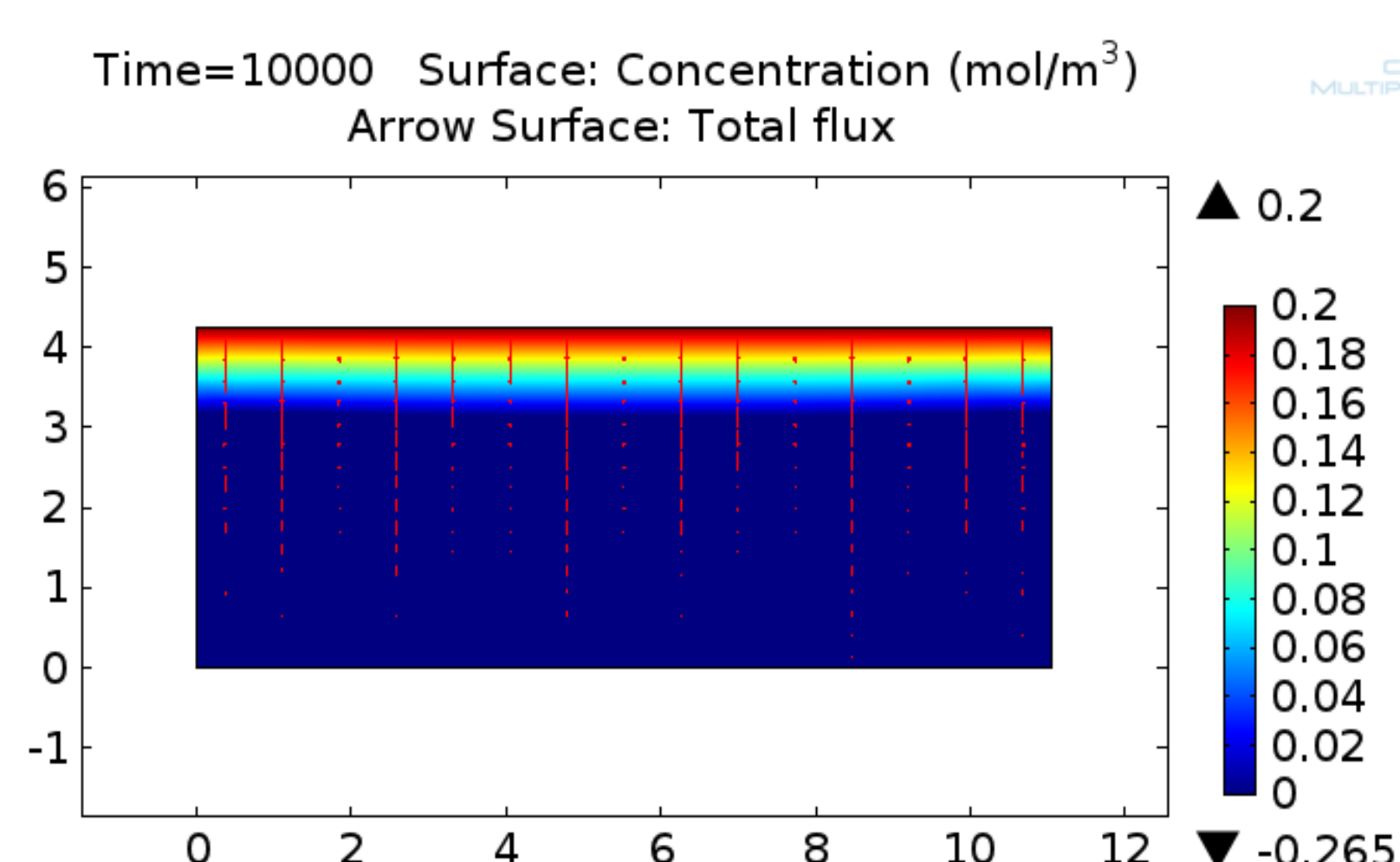


Figure 2. A 3D cell culture in a collagen scaffold. One million cells are seeded without an oxygenator. The cells towards the bottom of the culture would suffer from hypoxia.

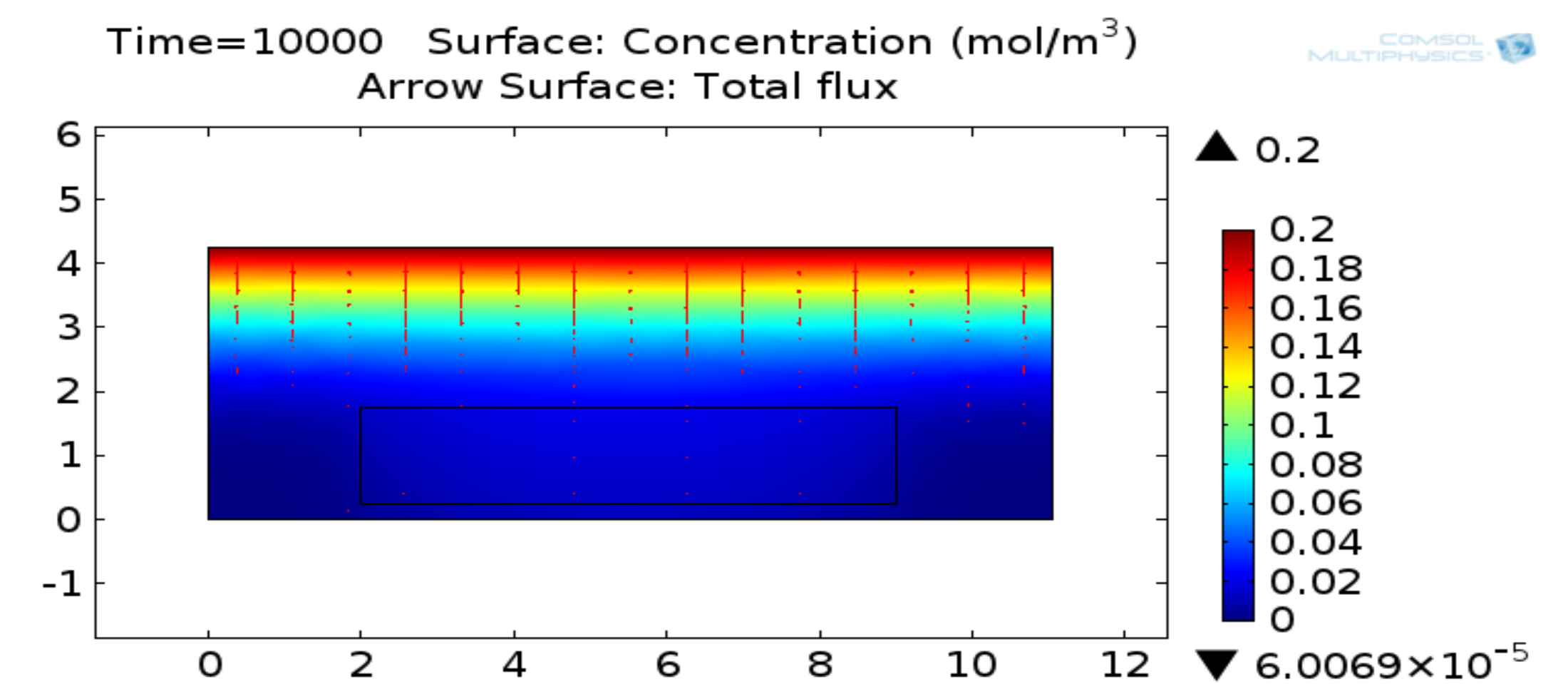


Figure 2. A collagen scaffold seeded with one million cells and with one O₂-release disk. The disk increased oxygen levels of the culture, however many cells will still experience hypoxia.

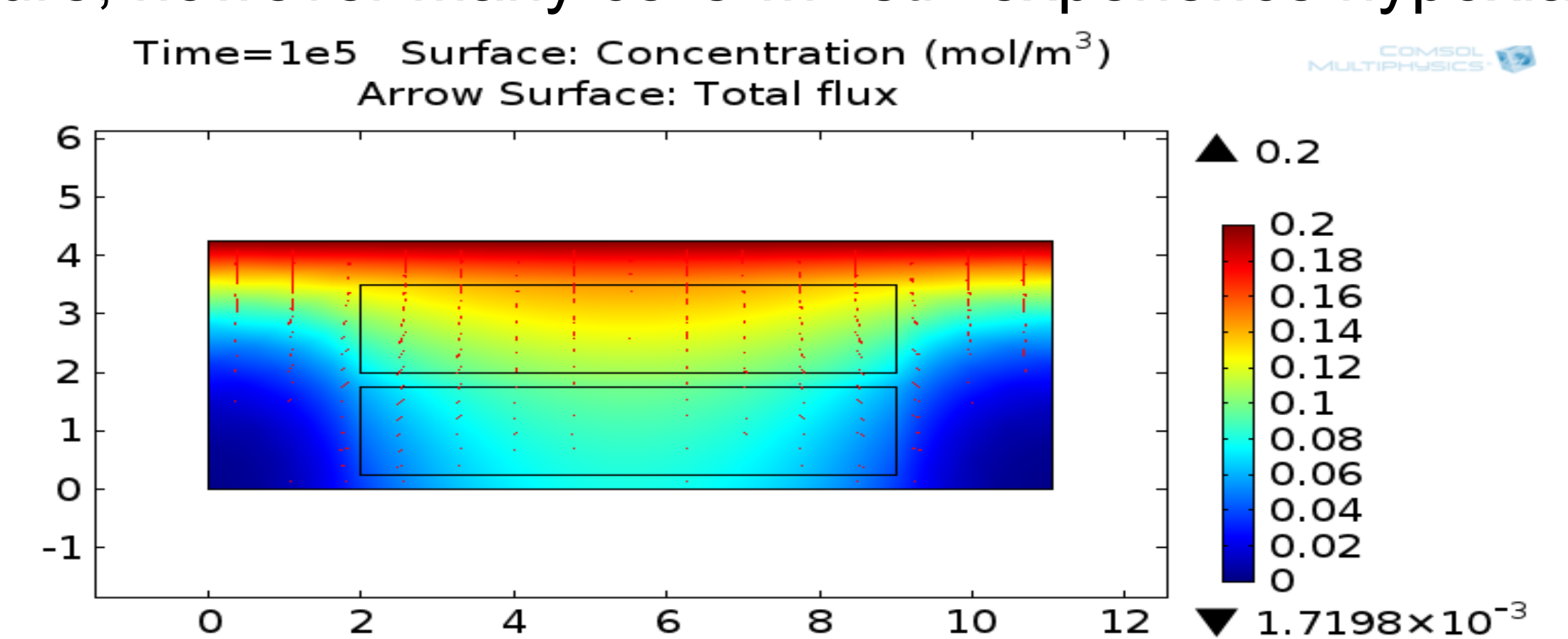


Figure 3. A collagen scaffold seeded with one million cells and with two O₂-release disks. The disks were able to effectively oxygenate the cell culture in order to prevent hypoxia

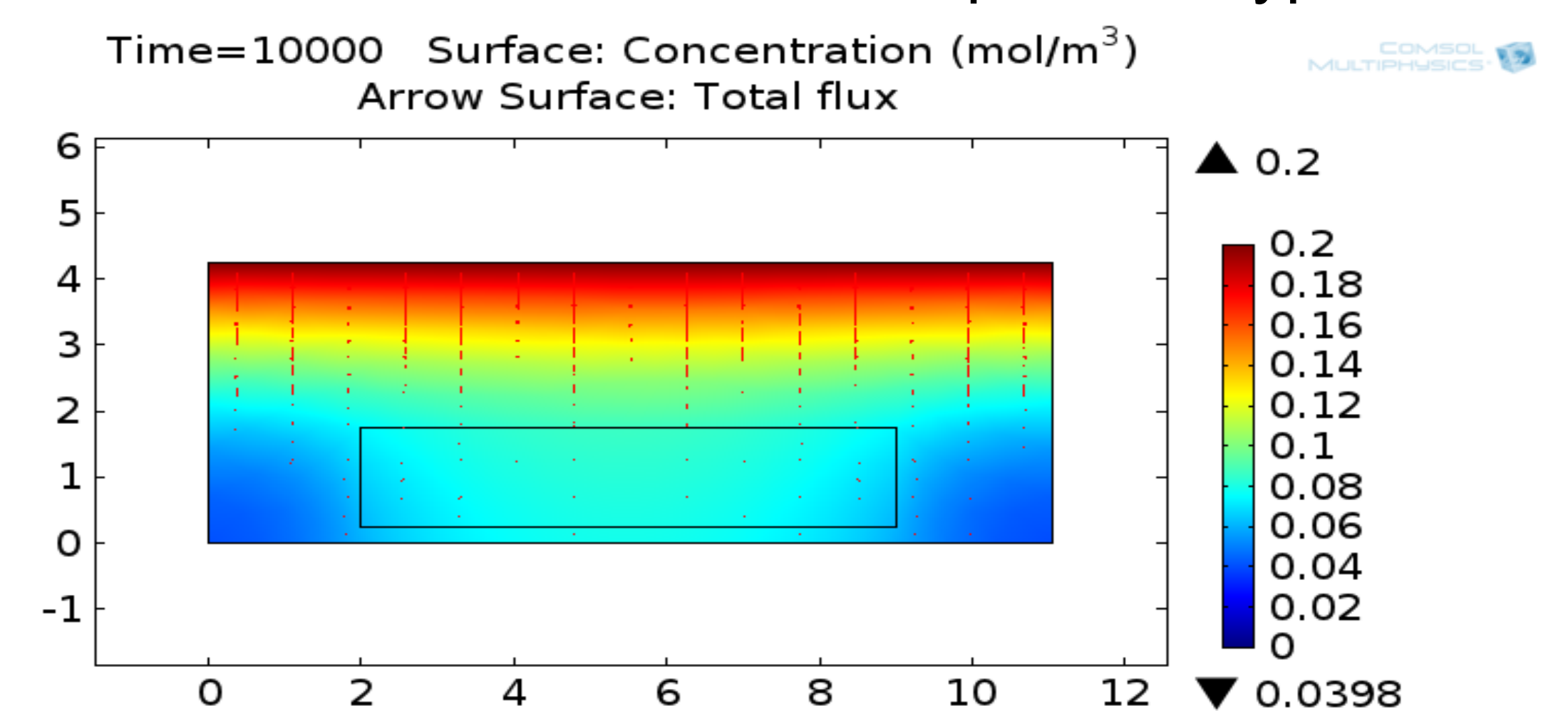


Figure 4. A collagen scaffold seeded with five hundred thousand cells and with one O₂-release disk. The disk was able to effectively oxygenate the cell culture in order to prevent hypoxia.

Conclusions: The results of the simulations demonstrated that a culture with one million cells requires additional oxygenation to avoid cell death. Cell death as a result of hypoxia occurs at an oxygen concentration less than 1e-4 mol/m³. One disk was not able to provide enough oxygen to avoid hypoxia in a culture of one million cells. However, the results showed if more disks were added to the cell culture or if the size of the cell culture was decreased then hypoxia can be eliminated. These results reveal that the addition of an oxygenator can be an effective method of providing oxygen to beta cells cultured in a 3D collagen scaffold.

References:

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