Submission ID #: 61928
Scriptwriter Name: Mithila Boche
Supervisor Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18888088

Title: Finite element modelling of a cellular electric microenvironment

Authors and Affiliations:
Miruna Verdes[1], Catherine Disney[1], Chinnawich Phamornnak[1], Lee Margetts[2], Sarah Cartmell[1]

[1]Department of Materials, Faculty of Science and Engineering, The University of Manchester
[2]Department of Mechanical, Aerospace and Civil Engineering, Faculty of Science and Engineering, The University of Manchester

Corresponding Authors:
Lee Margetts Lee.Margetts@manchester.ac.uk
Sarah Cartmell sarah.cartmell@manchester.ac.uk

Email Addresses for All Authors:
miruna.verdes@postgrad.manchester.ac.uk
catherine.disney@manchester.ac.uk
chinnawich.phamornnak@manchester.ac.uk
Lee.Margetts@manchester.ac.uk
sarah.cartmell@manchester.ac.uk

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes. Done.**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interview Statements are read by JoVE's voiceover talent.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 19
Number of Shots: 39 (38 SC)

Introduction

1. Introductory Interview Statements

NOTE to VO talent: Please record the introduction and conclusion statements

REQUIRED:

1.1. This method helps predict the electric microenvironment of a cell seeded onto a fibrous scaffold, such as the extracellular matrix **[1]**.

1.1.1. *4.4.1* (1:27-1:37).

1.2. There are two main advantages that arise from in silico modelling: the prediction of experimental conditions is 3D, while optimization is enabled by the ease of parameter change **[1]**.

1.2.1. *2.4.1* (0:09-0:13), *3.1.1* (0:09-0:18)

1.3. Electrical stimulation aids the regeneration of multiple tissues. This model and similar in silico models will help with the optimization of the stimulation parameters **[1]**.

1.3.1. *2.7.2* (0:17-0:29)

Protocol

2. Build the Model in COMSOL

2.1. To begin, open the **COMSOL** software and select **Blank Model**. In **Model Builder**, right click on **Global Definitions**, select **Parameters**, and add parameters according to Table 1 in the text manuscript. You can add them one by one or load them from a text file **[1]**.

2.1.1. SCREEN: 61928_screenshot_ 2-1.mp4. 0:04-0:44. *Video Editor: Speed this up if needed.*

2.2. In the **Model Builder** under **Global Definitions,** right click **Material** and select **Blank Material** to add materials **[1]**.

2.2.1. SCREEN: 61928_screenshot_2-2.mp4. 0:08-1:00. *Video Editor: Feel free to speed up the clip after 'culture media' is entered.*

2.3. To add material properties, go to the settings of the newly added material, then expand **Material Properties** and select **Electrical Conductivity** from **Basic Properties**. Press the '**plus**' symbol to add property. Repeat this process for **Relative Permittivity [1]**. Fill in the current material properties according to Table 2 from the text manuscript **[2]**.

2.3.1. SCREEN: 61928_screenshot_2-3.mp4. 0:09-1:11. *Video Editor: Feel free to speed up the clip after 'Electrical Conductivity' is entered.*

2.3.2. SCREEN: 61928_screenshot_2-3.mp4. 1:11- 2:48. *Video Editor: Speed this up.*

2.4. Next, left click on **Add Component** from the **Home** tab and select **3D** to add a new component node in the **Model Builder.** Again, right click on **Geometry**, left click on **insert sequence**, then double click on the **Full Model** and select the appropriate sequence **[1-TXT]**.

2.4.1. SCREEN: 61928_screenshot_2-4.mp4. 0:08-1:32. **TEXT: Sequence: RNC.** *Video Editor: Feel free to speed up the clip after 'full Model' is entered.*

2.5. Under the **Current** component node in the **Model Builder**, right click **Materials** and select **Material Link.** Associate materials for each component in this order: surrounding substance, coats, and cores **[1]**.

2.5.1. SCREEN: 61928_screenshot_2-5.mp4. 0:12-1:21. *Video Editor: Feel free to speed up the clip after 'surrounding substance' is entered.*

2.6. In the **Settings** tab for the surrounding substance, expand the **Selection** list to choose **Media Selection**. Expand the **Link** settings and choose the appropriate material like culture media from the dropdown list. To see the domains within the culture media block, activate the transparency button in the Graphics tab. Configure the other material links in the same manner **[1].**

2.6.1. SCREEN: 61928_screenshot_2-6.mp4. 0:06-1:31. *Video Editor: Feel free to speed up the clip after the 'transparency button' is activated.*

2.7. In the **Model Builder**, left click **Current Component**, select **Add Physics**, then expand the AC/DC module to select the **Electric Currents module** and click **Add to Component [1].**

2.7.1. SCREEN: 61928_screenshot_2-7.mp4. 0:08-0:35.

2.8. To define boundary conditions, select the **xy view in the Graphics tab [1]**. Go to the **Model Builder** again, right click on the **Electric Currents** node, and select **Ground [2]**.

2.8.1. SCREEN: 61928_screenshot_2-8.mp4. 0:09-0:27.

2.9. Next, keep the selection switch for the **Boundary Selection Active**, left click on the highest surrounding substance face parallel to the xz plane, and add **boundary 5** in the **Boundary Selection Box [1-TXT]**.

2.9.1. SCREEN: 61928_screenshot_2-9.mp4. 0:09-0:26. **TEXT: Scroll to select one of multiple overlaying surfaces**

2.10. In the **Model Builder**, right click on the **Electric Currents** node and select **Terminal [1].**

2.10.1. SCREEN: 61928_screenshot_2-10.mp4. 0:08-0:18.

2.11. Keeping the **Boundary Selection Active**, left click on the lowest surrounding substance face parallel to the xz plane and add **boundary 2** in the **Boundary Selection** Box **[1]**. Then, by expanding the **Terminal** section, select **Voltage in the Terminal** type dropdown list and fill in V0 for **Voltage [2].**

2.11.1. SCREEN: 61928_screenshot_2-11.mp4. 0:10-0.44.

3. Perform Simulation

3.1. Under **Global definitions** in **Model Builder**, left click **Parameters** and change the parameter **theta** to the fiber orientation angle desired for simulation **[1]**. Expand the component's node for each component in the **Model Builder,** then right click on **Geometry** and select **Build all [2].**

3.1.1. SCREEN: 61928_screenshot_3-1.mp4. 0:09-0:29.

3.1.2. SCREEN: 61928_screenshot_3-1.mp4. 0:41-0:58.

3.2. Left click the model root node in the **Model Builder** and open the **Add Study** tab, select **Stationary Study**, and right click **Add Study**. Under the newly added study, left click on **Step 1,** expand **Study Extensions**, check the Adaptive mesh refinement box, and click **Compute [3]** to obtain the refined mesh **[1]**.

3.2.1. SCREEN: 61928_screenshot_3-2.mp4. 0:05-2:27. *Video Editor: Feel free to speed up the clip.*

3.3. Left click the model root node in the **Model Builder** and open the **Add Study** tab, select **Stationary Study**, and right click **Add Study**. Under the newly added study, left click on **Step 1,** expand **Mesh Selection** and, select the mesh generated in the adaptive mesh refinement study **[1]**.

3.3.1. SCREEN: 61928_screenshot_3-3.mp4. 0:12- 0:39.

3.4. Proceed by right-clicking on the **Compute** button **[1]**.

3.4.1. SCREEN: 61928_screenshot_3-4.mp4. 0:06-0:39. *Video Editor: Feel free to speed up the clip.*

4. Analysis

4.1. Right-click on the **Results** node in the **Model Builder** and select **3D Plot Group** to edit **Settings**. Change the label to "**Charge density**" and select the **Parametric** study dataset by expanding **Data** set from the dropdown list. Then, in the **Color Legend**, check the boxes for **Show legends** and **Show maximum and minimum values [1]**.

4.1.1. SCREEN: 61928_screenshot_4-1.mp4. 0:06-0:37.

4.2. Again, under the **Results** node, right-click **Charge density** to select **Volume** and proceed to edit the **Settings** tab. Expand the **Data** tab, then select "**From parent**'' and fill in "ec.rhoq" in the Expression box. Check the Manual color range box from the **Range** tab and set the minimum and maximum to "minus 0.03" and "0.03" respectively **[1]**.

4.2.1. SCREEN: 61928_screenshot_4-2.mp4. 0:04-0:45. *Video Editor: Feel free to speed up the clip.*

4.3. Expand **Coloring and Style** and set **Coloring** to Color table and **Color table** to Wave. Check the **Color** legend box and **Symmetrize color range [1]**.

4.3.1. SCREEN: 61928_screenshot_4-2.mp4. 0:09-0:23.

4.4. Right click **Volume in Model Builder** and select **Filter. Go to the settings** tab and fill in the Logical expression for inclusion. Left click on the **Plot** button to visualize results in the graphics window **[1-TXT]**.

4.4.1. SCREEN: 61928_screenshot_4-2.mp4. 0.10-1:37. **TEXT: abs(ec.rhoq)>0.012**

Results

5. Results: Influence of Fiber Alignment on Electric Field

5.1. In this analysis, five different geometrical complexity stages that influence the simulation result are displayed **[1]**.

5.1.1. LAB MEDIA: Figure 3.

5.2. A mesh that is too coarse can hide relevant information **[1].** Using the adaptive mesh refinement, a mesh with smaller elements is obtained, as it is required for accurate results **[2]**.

5.2.1. LAB MEDIA: Figure 4. *Video Editor: Emphasize on left panel.*

5.2.2. LAB MEDIA: Figure 4. *Video Editor: Emphasize on right panel.*

5.3. At different levels of complexity for the fibrous mat model **[1]**, the strength of the electric field was influenced by the alignment of the fibers with respect to the potential gradient **[2]**.

5.3.1. LAB MEDIA: Figure 5. *Video Editor: Emphasize on "complexity" arrow.*

5.3.2. LAB MEDIA: Figure 5. *Video Editor: Emphasize on "fiber angle" arrow.*

5.4. Additionally **[1]**, the fiber alignment angle to electric potential gradient **[2]** impacts the space charge density in surrounding cell culture media **[3]**.

5.4.1. LAB MEDIA: Figure 6.

5.4.2. LAB MEDIA: Figure 6. *Video Editor: Emphasize on "fiber angle" arrow.*

5.4.3. LAB MEDIA: Figure 6. *Video Editor: Emphasize on charge density scale.*

5.5. In the scaffold fiber orientation study **[1]**, the steady state RNC model predictions were illustrated when fibers were parallel **[2]** or perpendicular to the electric field **[3]**. The charge density **[4]** and current density was influenced by scaffold fiber alignment relative to the electric field **[5]**.

5.5.1. LAB MEDIA: Figure 7.

5.5.2. LAB MEDIA: Figure 7. *Video Editor: Emphasize on left panel.*

5.5.3. LAB MEDIA: Figure 7. *Video Editor: Emphasize on right panel.*

5.5.4. LAB MEDIA: Figure 7. *Video Editor: Emphasize on light red and blue marks.*

5.5.5. LAB MEDIA: Figure 7. *Video Editor: Emphasize on arrows.*

Conclusion

6. Conclusion Interview Statements

NOTE to VO talent: Please record the introduction and conclusion statements

6.1. When changing model parameters such as theta, or material properties, the range for the resulting charge density may change significantly. For best visualization, the range must be optimized such that maximum variability in the charge density can be observed **[1]**.

6.1.1. *3.1.1 for "theta", 2.3.1 for "material properties", 4.2.1 for "resulting charge density"*