ANIMATING A NOVEL MECHANISM OF CELL MIGRATION: SIGNAL TRANSDUCTION EXCITABLE NETWORK (STEN)

by
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Abstract

Life is dynamic. Cells are constantly changing shape. Many do so by displaying a variety of protrusions that not only vary their appearance but also play a key role in important cellular activities such as cell migration, division, and phagocytosis. These protrusions manifest in unique shapes and sizes, ranging from finger-like filopodia to sheet-like lamellipodia.

It is well known that these protrusions drive outward from the cell body by a combination of actin polymerization and actomyosin-based contractions, referred to as “cytoskeletal activity”.

However, what determines the shape, and hence the identity of the protrusions, has remained a mystery until recently.

In recent years, a research team in the Johns Hopkins University Department of Cell Biology discovered a novel mechanism: Signal Transduction Excitable Network (STEN). It was found that STEN, a signaling network consisting of receptors, small GTPase proteins, and phosphoinositide lipids, determines the locations and lateral dimensions of cellular protrusions. Without STEN, cytoskeletal activity only produces transient, small extensions, or “puncta” which are ineffective in moving or reshaping cells. Manipulating the signal network can lead to alterations of the cytoskeletal system and morphing of the shape of the cell. Increasing or decreasing signal transduction activity can elevate or decrease

the speed and range of wave propagation respectively, converting pseudopodia into wider lamellipodia, or narrower filopodia.

This novel finding provides a direction for future biomedical research as it shows STEN plays a critical role in cell migration and morphology, and dysregulation of this system can lead to the development of a variety of diseases including cancer, and developmental and metabolic abnormalities.

However, the mechanism of STEN is difficult to succinctly explain due to its three-dimensional, dynamic nature. Current teaching materials are limited to simple line diagrams and crude confocal microscopy videos and photographs, none of which are adequate to allow for in-depth understanding of this intricate process.

To solve this challenge, I propose a narrative 3D animation that can help learners visualize and comprehend this novel mechanism. To maximize didactic efficacy, 2D images will be created to supplement the animation, and designed to be used independent of the animation if desired.

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Introduction

Cell Motility and Cytoskeletal Activities

Cell motility is a polarized process that involves cell shape change by formation of a protrusion from the leading edge and a retraction from the trailing edge. It underlies fundamental processes such as cell division, migration, phagocytosis, and certain pathological processes such as cancer metastasis and metabolic abnormalities (Miao, et al. 2019). Protrusions come in various of shapes, such as finger-like filopods, broader pseudopods, and sheet-like lamellipods. They are formed by dynamic assembly and disassembly of the most abundant and important of the cytoskeletal components: actin. At front of the cell globular actin (G-actin) polymerizes into actin filaments (F-actin) and generates mechanical force that leads to protrusion formation. At the rear of the cell, actin filaments interact with myosin II to form acto-myosin contractions, leading to traction force and retractions (Tang, Brennan 2017).

Other cytoskeletal components found in mammalian cells include intermediate filaments and microtubules, which will not be discussed in detail in this paper.

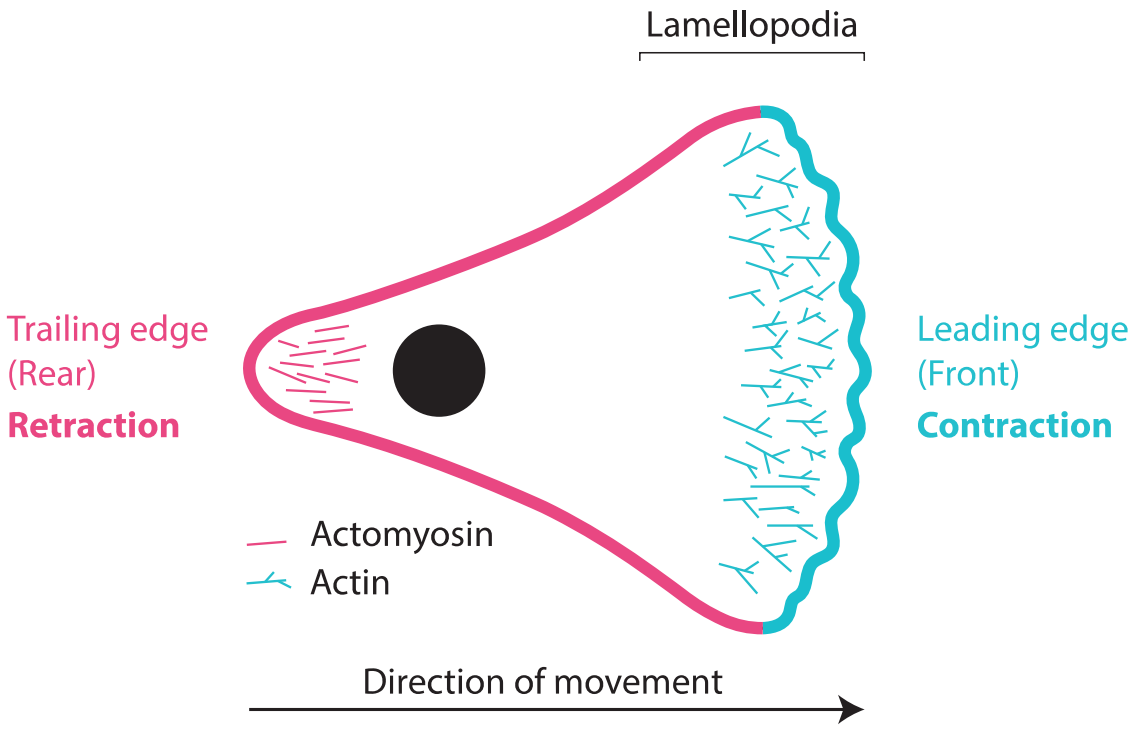


Figure 1. Schematic illustration of actin and actomyosin distribution. Lamellipodia are an example of cell protrusions that resemble a thin sheet. F-actin is found throughout the cell body but most prominently at the leading edge of the motile area. Branching and elongation of actin filaments promote protrusion. Established actin filaments interact with myosin II at the trailing edge and cause retraction. Actin polymers branch at 70 degrees (Pal, et al. 2019).

Recent findings have suggested that Signal Transduction Excitable System (STEN) is the mechanism that controls cytoskeletal activities, thus determining the number, location, and characteristics of protrusions and retractions.

Signal Transduction Excitable System (STEN)

Recent studies have found that STEN interacts with actin cytoskeletons to control dynamic wave patterns at the cell membrane. Its components include small GTPase proteins, lipids, and receptors, such as Ras proteins and phosphoinositides.

The signal transduction network can “fire” spontaneously, causing the cell to move in random directions. External chemical, mechanical, or electrical signals influence this spontaneous firing to guide the cells and direct their migration. “Front” molecules such as Ras and PIP3 organize at the cell’s front leading to actin polymerization and protrusion, while “back” molecules such as PTEN and PIP2 dissociate from the protruding regions and organize at the rear of the cell leading to acto-myosin interaction and contraction (Parent, et al. 1998) (Kriebel, et al. 2008). Such complementary accumulation of front and back components is crucial in organizing cell motility activities (Cai, et al. 2010). Visualization with fluorescent proteins and lipid biosensors demonstrate that these molecules shuttle on and off the membrane sequentially like a laterally moving stadium wave (Miao, et al. 2019).

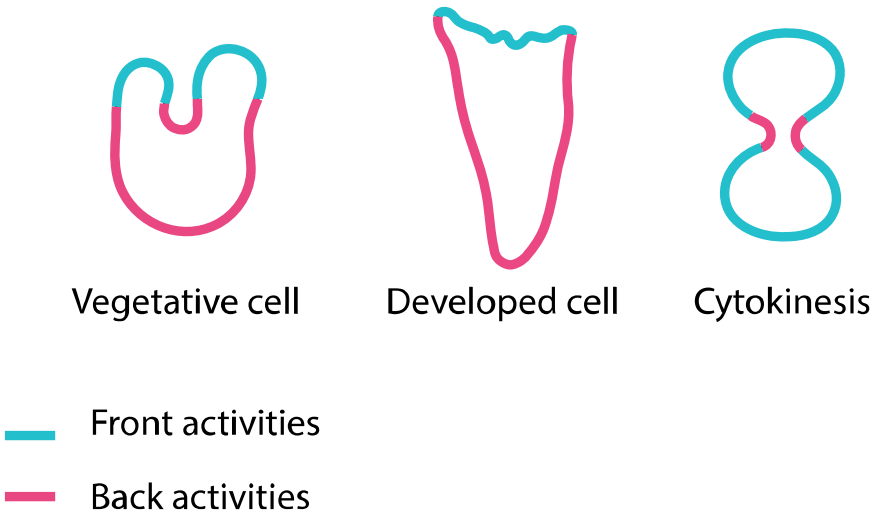


Figure 2. Complementary distribution of front and back molecules in cells during morphological changes. *Image adapted from Pal et al, 2019.* Front activities such as Ras or PI3K activation are seen at the protruding edges of the cells (denoted in blue). These are complemented by back activities such as PTEN dissociation and cellular retractions (denoted in pink).

Altering signal transduction activity can elevate or decrease the speed and range of wave propagation and convert a pseudopod to a wider lamellipodia or narrower filopodia (Miao, et al. 2019).

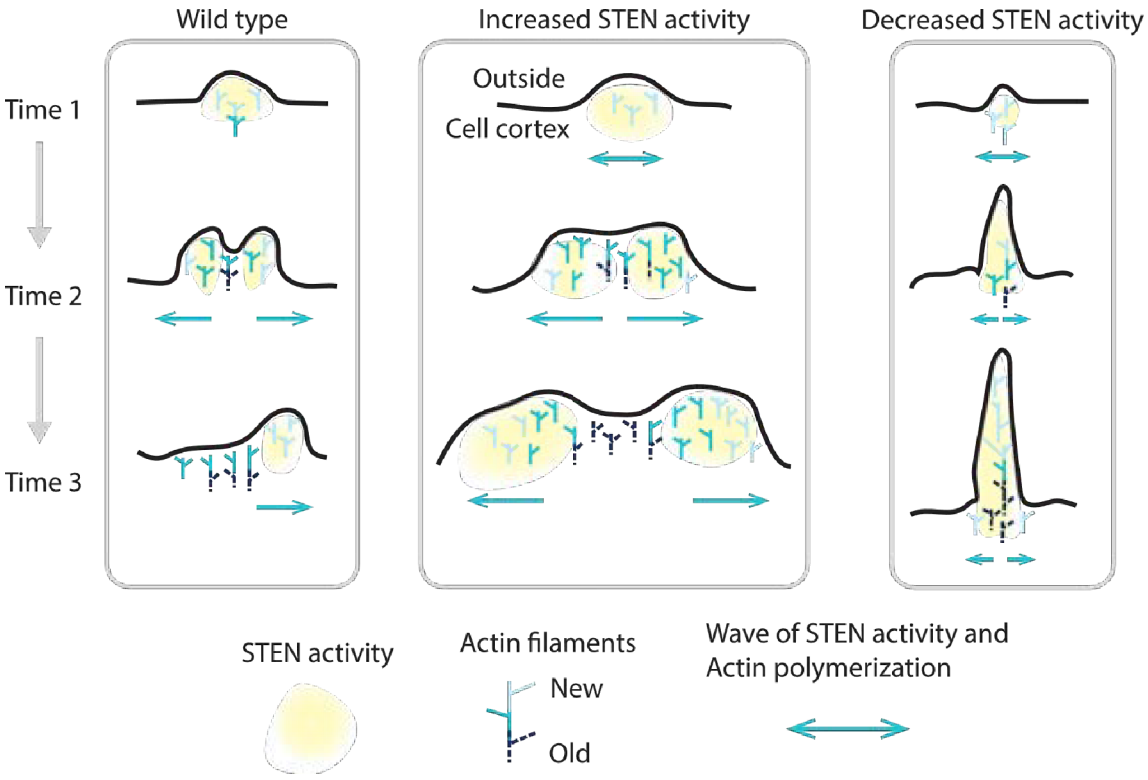


Figure 3. STEN wave propagation and protrusion formation by actin polymerization. *Image adapted from Miao et al, 2019.* STEN activity propagates along the cell membrane like a lateral stadium wave that leads to the activation of cytoskeletal activities. When STEN has moved away, actin polymerization also ceases and existing filaments break down (Miao, et al. 2019). Increasing STEN activity leads to elevation in speed and range of wave propagation, resulting in lamellipodia-like structure. On the other hand, decreasing STEN activity leads to reduction in speed and range of wave propagation, resulting in long protrusions such as filopodia.

This network contains several tumor suppressors, oncogenes, and other factors implicated in normal development and metabolic abnormalities. Therefore, understanding this delicate system is crucial to understanding morphological development and associated pathological mechanisms. This project will focus on the creation of a 3D animation to demonstrate the intricate mechanism of STEN and its interplay with cytoskeletal activities. This project will further explore STEN's significance in normal and pathological development.

Significance of Cell Motility

i. Immune Regulation

Cell migration plays a key role in inflammatory responses when leukocytes are directed through tissues and vessels towards chemoattractants and invading pathogens. It also guides cells to lymph nodes and promotes interactions between leukocytes and lymphocytes (Nourshargh, Alon 2014).

ii. Embryogenesis and Nervous System Development

During embryogenesis, carefully directed migration of individual or groups of cells lead to organogenesis and nervous system formation (Montell 2008). Notable examples are the coordinated movement of epithelial cells at the beginning of gastrulation and neurulation (Theveneau and Mayor 2012), migration of primordial germ cells across the embryo towards the somatic gonads (Richardson and Lehmann 2010) and glial and neural cell movement in the peripheral nervous systems (Klambt 2009).

iii. Tissue Regeneration

Fibroblasts and keratocytes display organized group motility to perform tissue regeneration during wound healing (Shaw and Martin 2009).

iv. Cancer Metastasis

Metastasis, when tumor cells detach from their primary site and spread to another organ, also requires orchestrated cell migration. Four key steps are involved in metastasis: invasion, intravasation, circulation, and extravasation. (Valastyan and Weinberg 2011).

The invasion is directed by chemoattractants from nearby blood vessels and macrophages. The tumor cells first display protrusions called invadopodia, which secrete metalloproteases to degrade and penetrate through barriers to escape into the circulatory system. For intravasation and extravasation processes, tumor cells display behaviors that mimic those of leukocytes when they migrate in and out of the blood vessels in response to inflammatory cues (Friedl and Wolf 2004).

The animation associated with the project will focus on extravasation and colonization.

Existing Teaching Tools

The concept of interplay between cytoskeletal activities and signal transduction excitable network is a novel finding that has proven difficult to visualize. Current teaching tools are limited to schematic, crude, 2D diagrams that are insufficient to fully explain the intricate three-dimensional nature of the mechanism.

Currently available 3D animations are focused only on cytoskeletal activities, usually limited to the actin filaments. The interactions between the signaling molecules, cell membranes, and cytoskeleton including both actin and myosin will be visualized through this project for the first time.

3D Animation as a Learning Tool

It is well said: “a picture is worth a thousand words”.
Studies have shown that animation is highly effective in helping learners to memorize, attend, store, and retrieve new information as it helps visualize dynamic processes that would otherwise be difficult to comprehend. The use of 3D assets makes the learning process more effective by providing temporal and spatial information with minimal loss of detail
Bellezza (1985) demonstrated that learners were able to recall information better when presented with visuals and text rather than text alone because visuals are encoded as intact images in long term memory. This will be discussed more in-depth in the next section, *Learning Theory*. The more complex a subject is the greater the benefit of 3D animation. Visualization through 3D animation is ideal

for this topic because it promotes a deep understanding of the complex mechanisms involved in STEN.

Learning Theory

Learning theories are useful guides for the development of visual media to teach large amounts of complex information. Several learning theories were explored, and various aspects were employed in the design of this animation.

Cognitive Theory of Multimedia Learning devised by Mayer (2014) proposes that most effective learning occurs when learners can make connections between corresponding verbal and visual representations in working memory. To maximize this point, animation key frames were timed to closely correspond with narration and labels.

Similarly, Information Delivery Theory proposes that multimedia learning is maximized when information is presented to the learners in multiple ways simultaneously (Pollock, Instructional design strategies and tactics 1992), including informal narration, written text, music and sound effects. In accordance with this theory, a variety of sound effects and background music were implemented.

Cognitive Loan Theory by Pollock (2002) states that information should be presented in a way that reduces the cognitive load on working memory, which animation, as a sequence of motion pictures with a narration, can do (Rieber 1990). In addition, Elaboration Theory (Reigeluth 1979) was employed to reduce working load on the brain.

As the theory instructs, a fully solved result, the “big picture” that gives a general overview of the subject matter, was presented first then elements were gradually introduced so that learners are able to absorb new information in small amounts. The animation first depicts an event that triggers a cascade of cellular responses– a hand that gets pricked by a blackberry thorn. It is a common injury that many viewers will be able to relate to without the need for additional scientific background knowledge.

Then examples of cell motility processes, such as migration, division, and phagocytosis are presented, without an explanation of the underlying mechanisms. Intuitive visuals accompany these examples which make the animation smoothly transition into the next step without introducing unnecessary cognitive load on the audience. Then the concept of protrusions is introduced, followed by cytoskeletal activity. Both are topics the target audience, cell biology and medical students, should already be familiar with. Finally, the focus of this animation, the mechanism of STEN, is explained.

All layers of information are presented in the order of complexity.

Order of Presentation of Key Scenes in Animation

1. A break in the epidermal layer is created by a thorn on a blackberry bush.

2. A neutrophil is seen changing its shape as it extravasates from a blood vessel and migrates to the site of injury, attracted by inflammatory cytokines.

3. Examples of cellular processes where cell motility plays a crucial role are presented.
    i. Migration: camera zooms into a migrating neutrophil
    ii. Division: a group of Streptococcus epidermidis, skin commensal bacteria, breaches the skin via injury site and undergoes division.
    iii. Phagocytosis: neutrophils reach the target site and engulf Streptococcus epidermidis.

4. Scene changes and a non-specific cell is seen crawling on an epithelial cell layer, changing its shape from finger-like filopodia, broader pseudopodia, and sheet-like lamellipodia.

5. The view zooms in on the cell. Cytoskeletal activity is shown, including actin filament polymerization and actomyosin-based contractions.

6. A protrusion shown in an earlier scene regress into a puncta, or a ruffle, as the cytoskeleton disintegrates, demonstrating why cytoskeletal activities alone cannot produce effective cell motility.

7. Signal Transduction Excitable Network (STEN) is introduced. Its interaction with cytoskeletal activities to determine the locations, dimensions, and characteristics of cell protrusions is explained.

8. Examples of life processes where the interactions between STEN and cytoskeletal activities play a vital role are presented.
    i. Embryogenesis: embryo development is depicted from 7th week to full term.
    ii. Nervous system wiring: the nervous system in a full-term baby is depicted.
    iii. Cancer metastasis: cancer cells extravagate from a blood vessel and develop metastatic colonies.
    iv. Immune regulation: animation reverts to earlier scene of the break in the epidermal layer. The camera zooms out to reveal a wide view of the scene.
    v. Wound healing and tissue regeneration: camera zooms further out to reveal a wide view of the injury during and after the process of healing.

Intended Audience

The primary audience of this project consists of biology undergraduate and graduate students as well as medical students. The secondary audience includes researchers in relevant fields and the general public with an interest in current scientific topics. Educators will be able to use the animation and images to supplement other teaching materials. The animation will have an introduction to the principles of cell migration so that those with no prior knowledge of this area will be able to comprehend and appreciate the novel influence of the STEN mechanism.

Project Objectives

1. **Visualize complex cell migration mechanisms**. The first part of the animation will introduce conventional cell migration mechanism – cytoskeletal activity, after which the topic of signal transduction activity will follow. The animation will demonstrate that STEN interacts with cytoskeletal activity to determine the dimensions and locations of cellular protrusions, which play a critical role in cell migration and morphology. The animation will be supplemented by didactic 2D images, which may be used independently if desired.

2 **Educate the viewers with narrative 3D animation supplemented by didactic 2D images**. An animation with both 2D and 3D assets in conjunction with narration and sound effects will be created to maximize learning experience for the viewers.

3. **Provide an accessible visual resource that will enhance understanding of the mechanism and suggest further research directions**. For easy access, the animation will be posted online.

4. **Captivate viewers with the beauty of cell biology and entice them to investigate the subject further.** The animation will be rendered in a cinematic and visually appealing style to be both educational and entertaining.

Potential Contribution to Public Health and Biocommunication

This project will highlight the pivotal role of biocommunication plays in helping researchers translate complex findings into effective teaching tools for primary education. This project will also exemplify that the role of Medical Illustrators is not limited to education, but also extends to the advancement of biomedical research practices and promotion of innovative ideas that inform the direction of future research.

Materials and Methods

Research

Literature including books and published peer-reviewed scientific papers, and data provided by the content expert were extensively reviewed. In addition to cytoskeletal network and signal transduction, topics including immune regulation, skin physiology, embryogenesis, tissue regeneration and metastasis were researched for accurate representation in 3D. Regular meetings were held with the preceptor to verify accuracy of the content.

Current teaching materials were also evaluated to gauge the level of available educational tools. Previous 3D animation on the subject was limited to depicting cytoskeletal activities. No material was discovered by the author depicting STEN. Existing teaching materials related to STEN were limited to crude 2D schematics.

Software Overview

Several programs were used to create the 3D animation. Adobe Photoshop and Procreate were used to generate storyboards. Adobe After Effects was utilized to create the animatic. Adobe After Effects was also used to composite final 3D renders and to complete post-process editing. Pixiologic ZBrush and Cinema4D were used to build 3D assets. Quixel Bridge was used for the creation of background scenes and distant low-resolution assets. Redshift, a third-party

GPU-based rendering engine, was utilized for materials, lighting, and camera movements.

Script and Narration

After initial research was complete, a script was developed with a maximum of 350 words to limit the duration of the animation to approximately 3 minutes. The script was revised multiple times to ensure the animation was as succinct and focused as possible and to captivate the audience's attention for the duration of the animation. Extra consideration was given to this in light of the emergence and popularity of time-limited social media platforms such as Youtube, Twitter and TikTok.

The appropriate level scientific vocabulary was used for the target audience (undergraduate and graduate biomedical students, and medical students). The script was checked by both the thesis advisor and content expert/preceptor for accuracy and clarity. 12 revisions were made before the script was deemed final. Storyboards and an animatic were produced. A professional voice-over artist was hired and a voice-over track was recorded.

Storyboard

The finalized script was broken down to a sequence of scenes. Thumbnail sketches with basic colors were developed for each scene using Adobe Photoshop and Procreate. Several revisions were made until approved by the advisor and preceptor.

Animatic

Once the script and storyboard were confirmed, images from the storyboard and a rough narration were composited in Adobe After Effects into an animatic. The animatic was used to check the flow of the animation with the narration, as well as to better visualize camera movements and transitions.

3D Asset Creation

Rough sketches were developed using collected references such as photographs, scanning electron micrograph (SEM) images, schematic diagrams from research papers, and molecular structure data from the Protein Data Bank (PDB). Pixologic ZBrush and Cinema4D were used to model 3D assets with plug-ins including ePMV, X-Particles, Quixel Bridge, and Redshift Renderer.

i. Blackberry Branch

Fruit

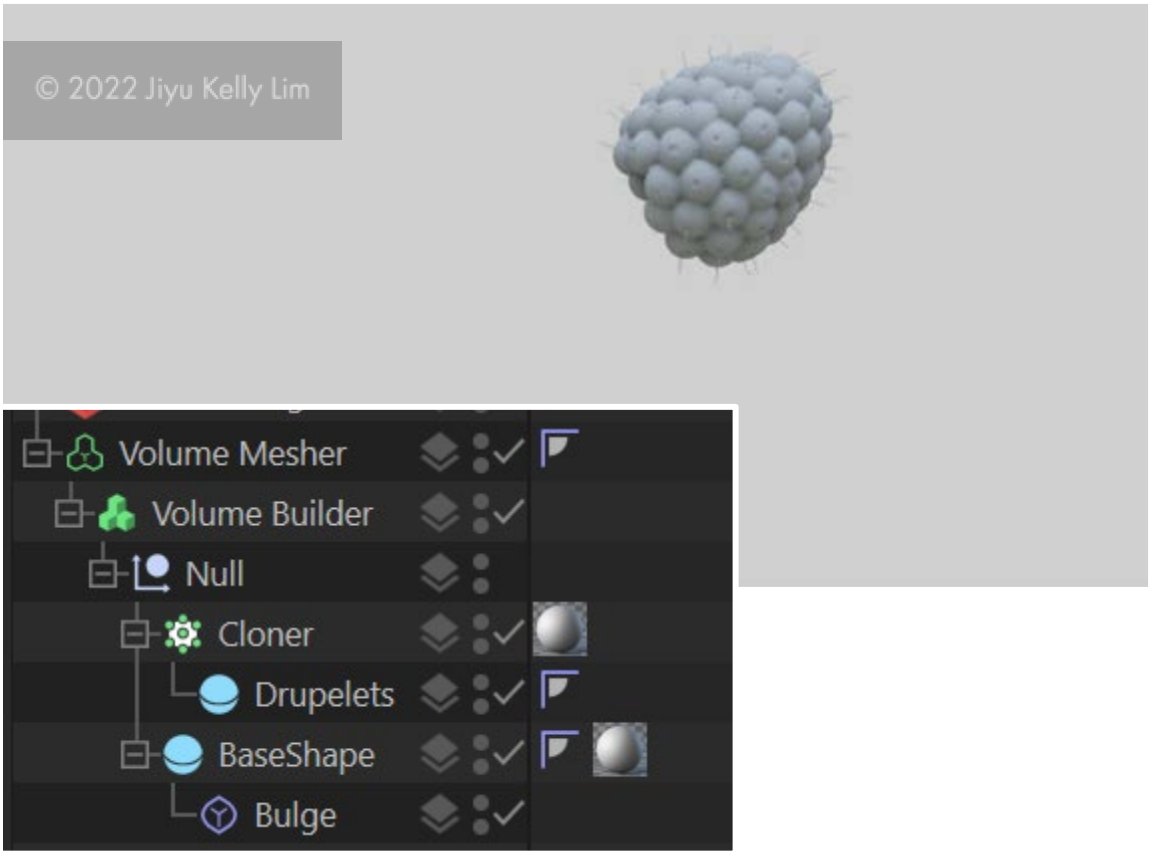


Figure 4. Blackberry fruit model and its layers in Object Manager.

An icosahedron sphere was created and a **Bulge** deformer used to form the base shape of the blackberry fruit. Smaller spheres were created and placed in a mograph **Cloner** in Object Mode, with the icosahedron set to Object. The cloner was made editable then placed within **Volume Builder** and **Volume Mesh**. To reproduce styles, short and sparse **hair material** with a slight kink was added to the fruit object's polygon surfaces. The **Magnet** tool was used to create pits where the styles arise from the fruit.

Branch with Thorns

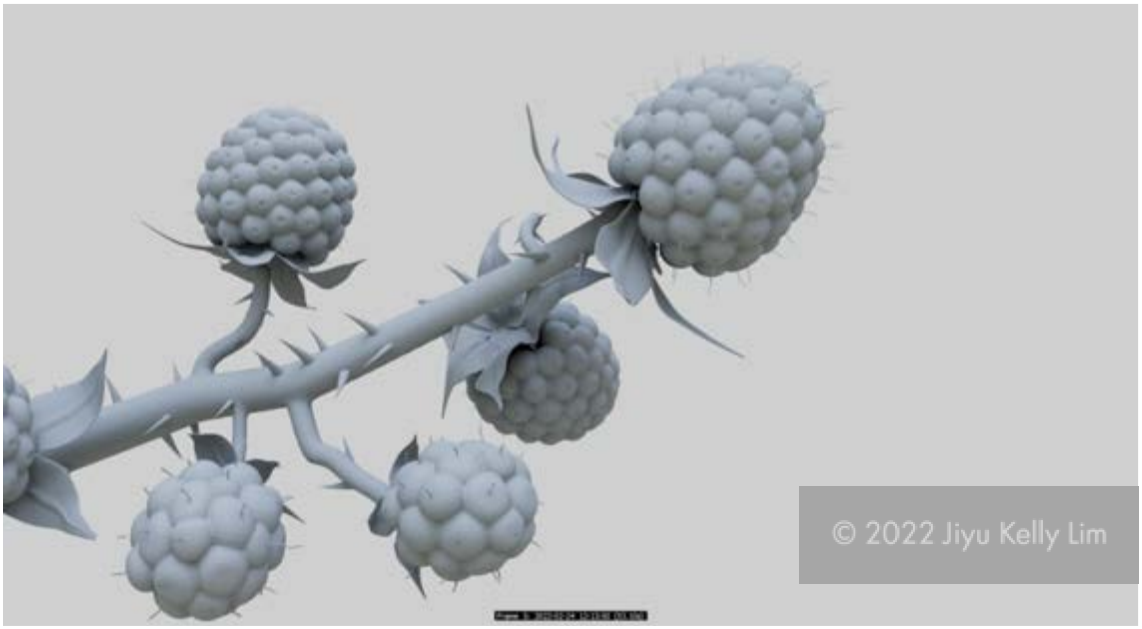


Figure 5. Blackberry fruit with branch and thorns added. Text not intended to be read.

The base shape of branch was drawn with the **Spline Pen** tool, which was placed within a **Sweep** generator with a circle and then made editable.

To create thorns, a pyramid object was created then modeled into a thorn shape with the magnet tool, and placed within a mograph **Cloner** with Object Mode set to Branch. A **Random** Effector on the X axis rotation was applied to give varying radial placement to each thorn.

Background assets

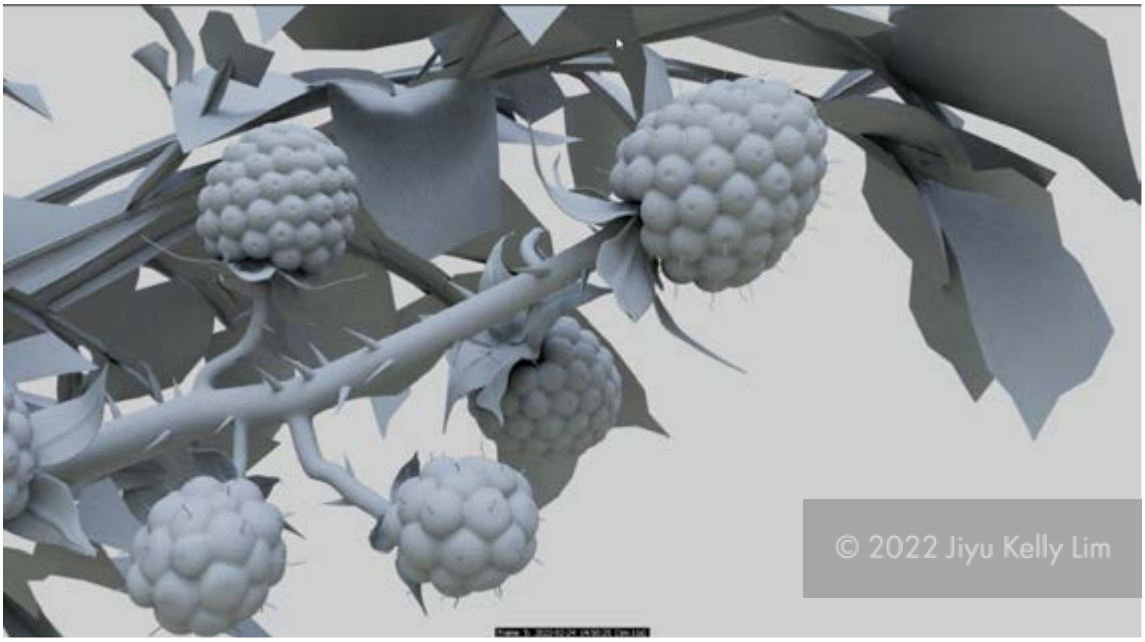


Figure 6. Blackberry fruit, branch, and thorns, with additional branches behind the main figure. Note The leaves lack details in shape until texture has been applied. Text not intended to be read.

Figure 7. Blackberry branches after texture has been applied. Once texture has been added, details in leave shapes are shown. Text not intended to be read.

Figure 8. Quixel Bridge is used to import a blackberry bush model.

Quixel Bridge was used to create secondary branches in the background that do not require close-up views. An appropriate blackberry bush model was selected then downloaded and imported to Cinema4D. It was scaled up and placed so that only a few branches are seen by the camera, not the whole plant.

ii. Skin Environment

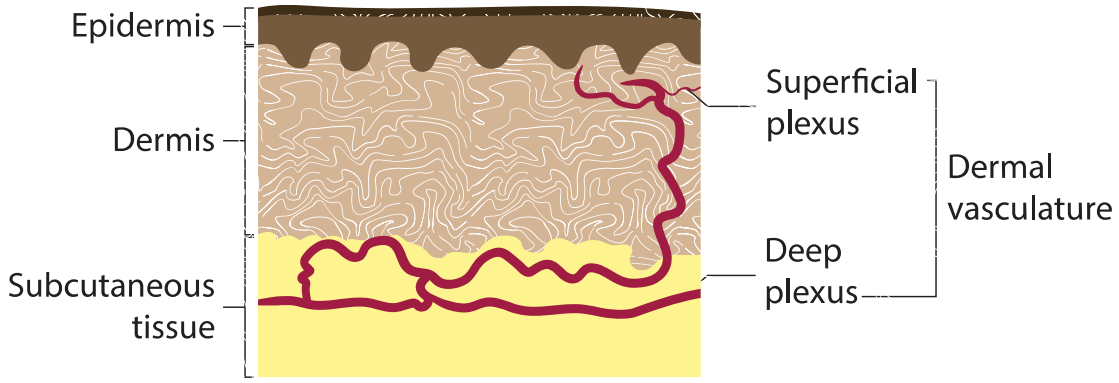


Figure 9. Skin cross-section. *Image has been adapted from Berger and Elston, 2006.*

The skin is the largest organ of the body. It performs numerous vital functions, from protection from external assailants to thermoregulation (Kolarsick, et al. 2011). Most of these functions require careful cell migration, which is why the main scene of the animation takes place within the skin environment.

The skin cross-section is divided into epidermis, dermis, and subcutaneous tissue. Vessels are found in subcutaneous and dermal layers. The outermost layer, the epidermis, is comprised of cells called keratinocytes. The middle layer, the dermis, contains a variety of cells and structures such as sweat glands and nerves, embedded in an extracellular matrix primarily made up of collagen, the most common type of structural protein found in the body. The innermost layer called the subcutaneous layer and is made up primarily of small lobes of fat cells, also called lipocytes (Kabashima, et al. 2019).

For this project, dermal and subcutaneous tissue layers were created, which required the modeling of vessels, lipocytes, and collagen. Two types of collagen of different thicknesses were created. Type I collagen, the thickest type, is the most abundant collagen found in the dermis (Burgeson 1982). Type IV collagen, a major component in of the dermal-epidermal junction, has a honeycomb pattern in contrast to other major collagen types which are fibrillar (Stage 1982).

Blood Vessel

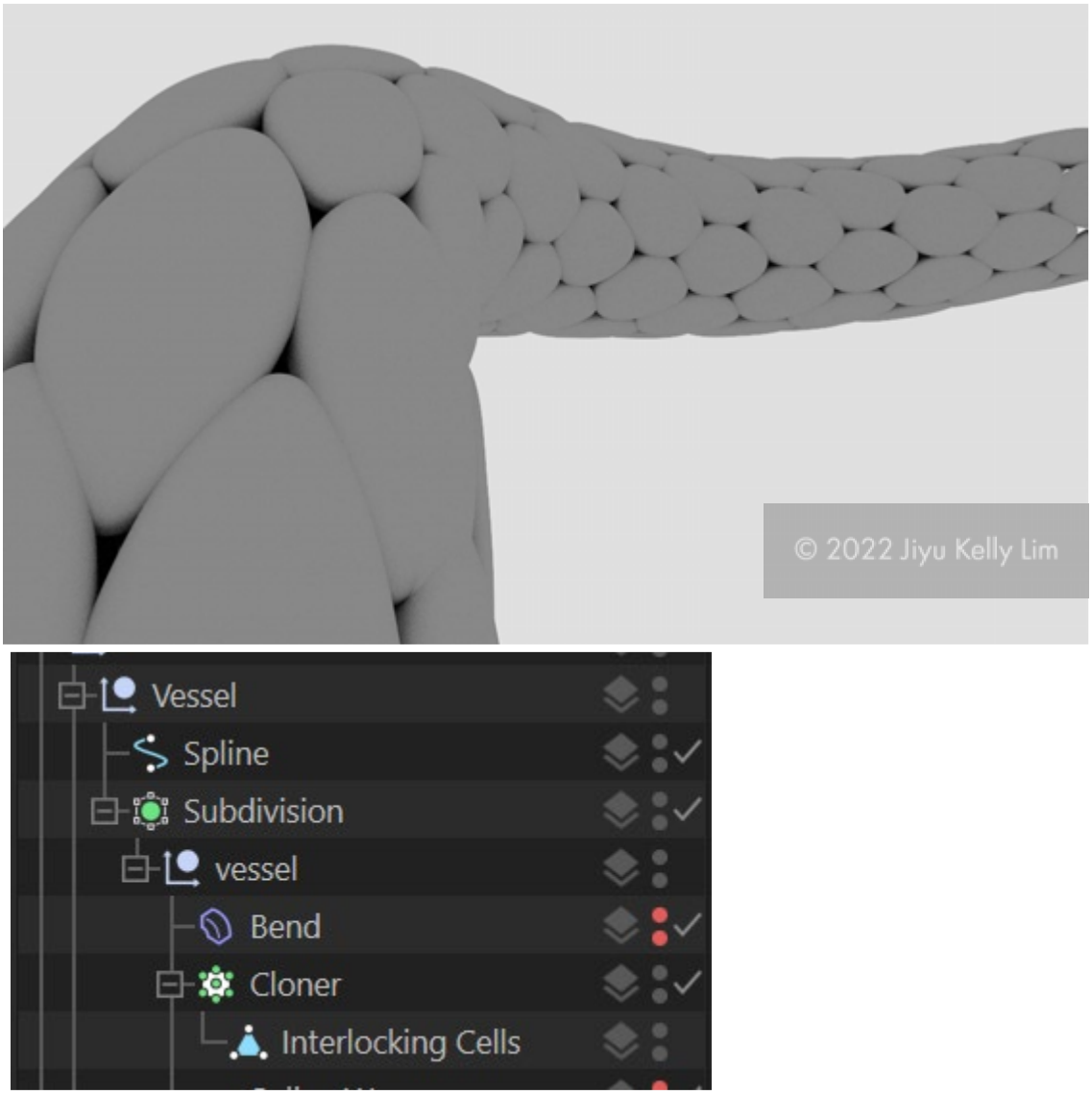


Figure 10. 3D model of a blood vessel and its layers in Object Manager.

Two interlocked cells were modeled from a cube then made into a single object by **Connect and Delete**. The object was placed within a mograph **Cloner** and Mode was set to Grid Array. Count was changed to 8 x 1 x 8 and the size adjusted to personal preferences. The model was then placed within a **Bend** deformer with enough strength to create a tube shape.

Lipocytes

Figure 11. Lipocyte models.

Three different shapes and sizes of lipocytes were created using spheres, **Displacer** deformer, and the **magnet** tool. The different versions of lipid molecules were placed within a **cloner** with **displacer** set to World to add diversity to the shapes and sizes. **Rigid body** tag was added to the lipid molecule models. An invisible box was created to constrain the placement of the lipid molecules, and **Collider Body** tag was added. An mograph **Emitter** was placed as a parent of the lipid and activated to generate a number of lipid molecules within the invisible box.

Type I Collagen

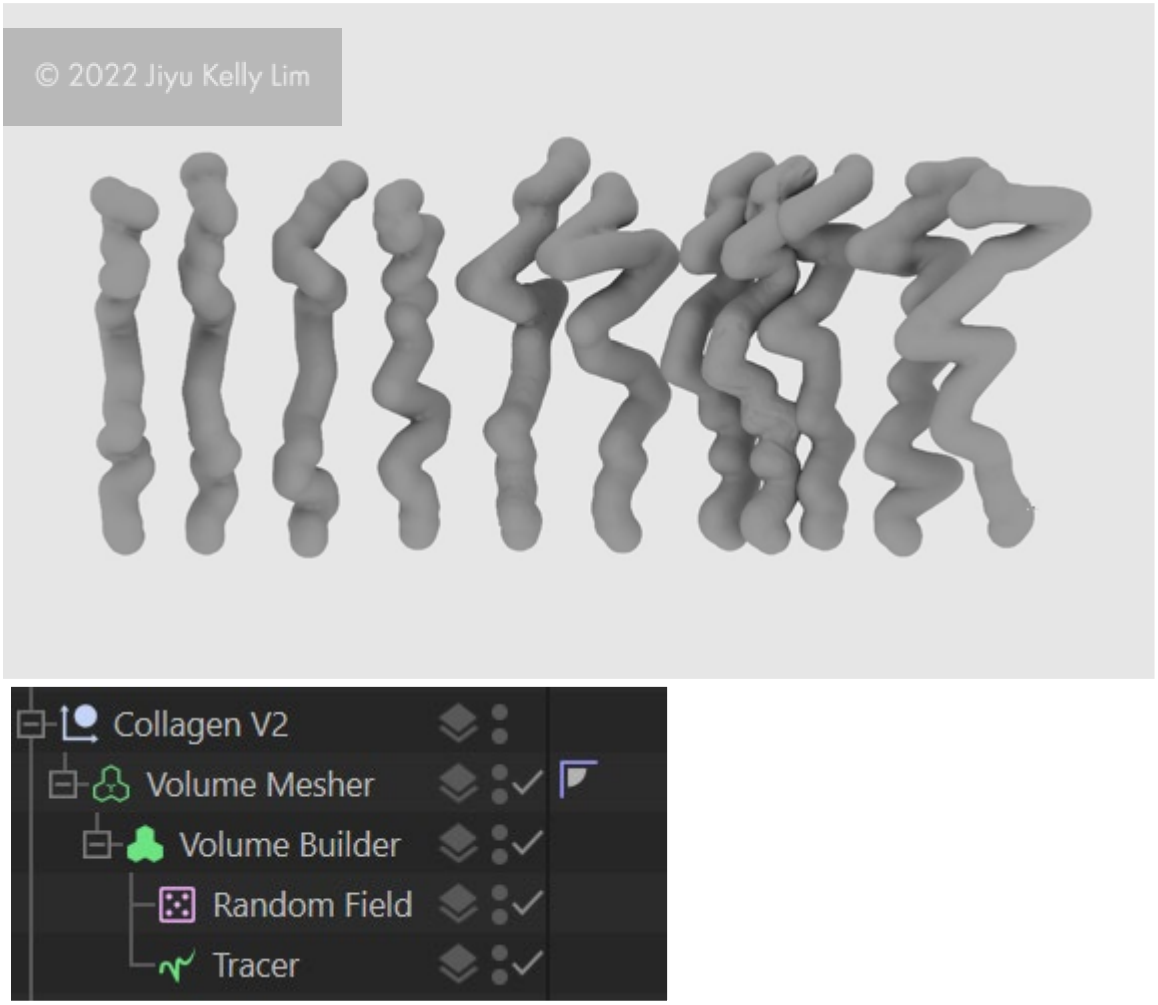


Figure 13. another collagen model and its layers in Object Manager.

A mograph Matrix was moved in the Y-axis by 200 cm by **Plain** Effector. Randomness is added by **Random** Effector set to turbulence mode. Parameters of the **Random** Effector were adjusted to create an organic shape. The movement in Y-axis were made into shapes by **Trace**r placed within **Volume Builder** and **Volume Mesher**. Random Field was added under **Volume Builder** to create more unevenness in the geometry.

Type IV Collagen

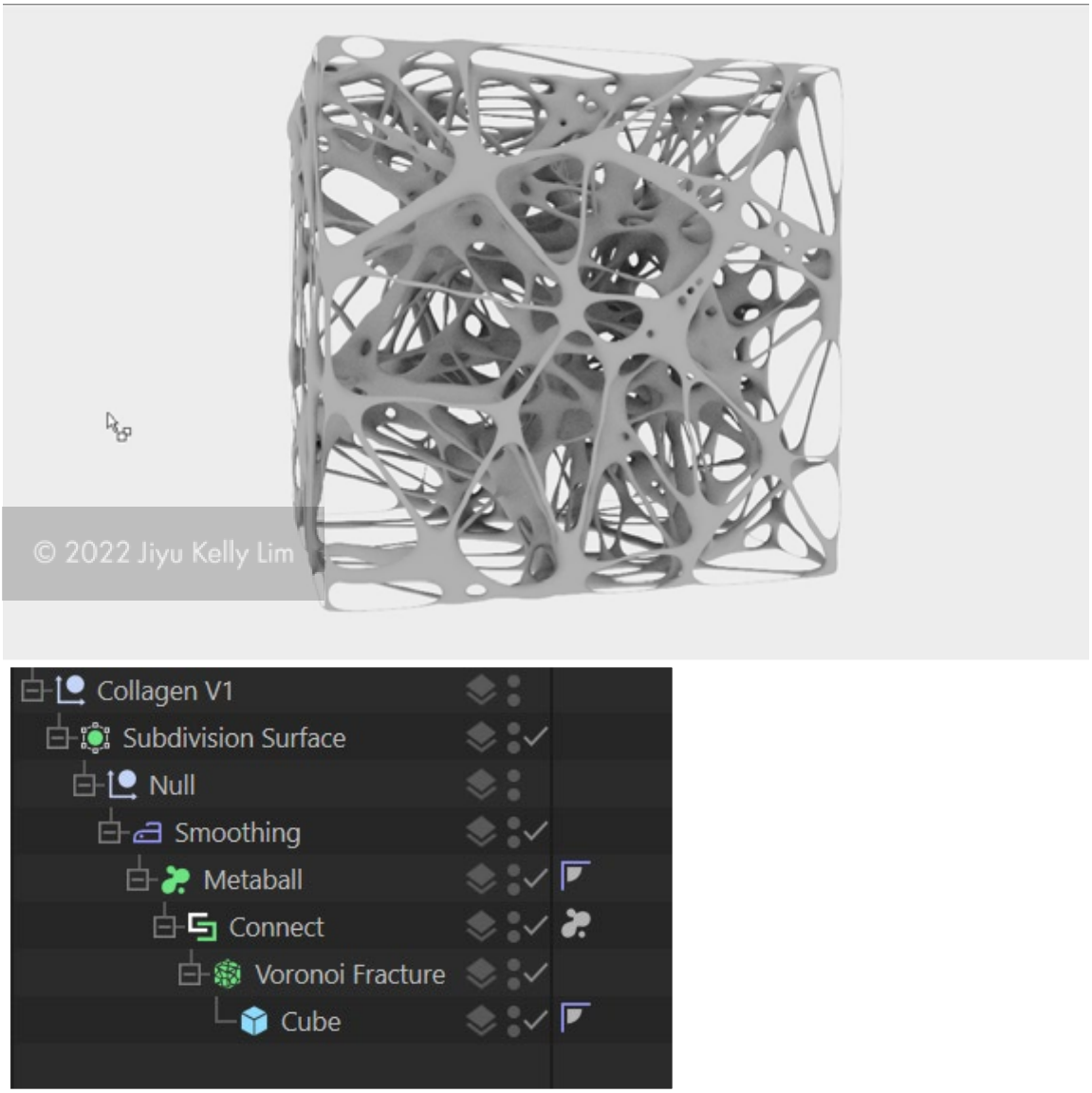


Figure 12. Collagen model and its layers in Object Manager.

A cube was placed within **Voronoi Fracture** and then placed within **Connect** and **Metaball**. Finally **Smooth Object** was applied to create an organic look.

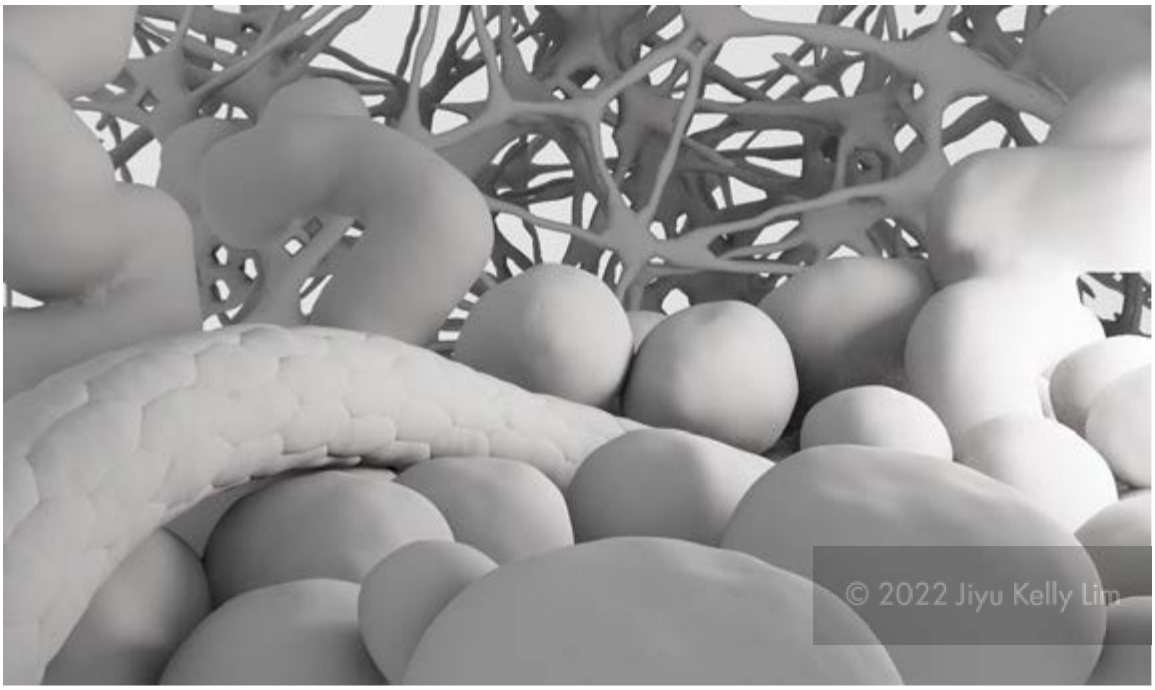


Figure 14. Skin environment. Multiple copies of both types of collagens were arranged carefully to mimic a skin environment: dermis and the subcutaneous tissue, with blood vessels running through.

iii. Leukocytes and Bacteria

Neutrophils and macrophages are the main leukocytes that play a role in the acute inflammatory response to assaults to the skin. Neutrophils are the first cells to arrive at the wound bed and remain for approximately 24 hours before undergoing apoptosis. They kill microbes and promote wound healing. They also secrete a variety of cytokines that attract other leukocytes such as monocytes, which arrive at the injury site 5-6 hours postinjury.

Monocytes differentiate into macrophages, which are the key immune cells in the wound healing process. They secrete anti-inflammatory pro-repair cytokines that promote wound healing and tissue regeneration by fibroblasts and keratinocytes (Larouche et al, 2017; Samantha et all, 2018).

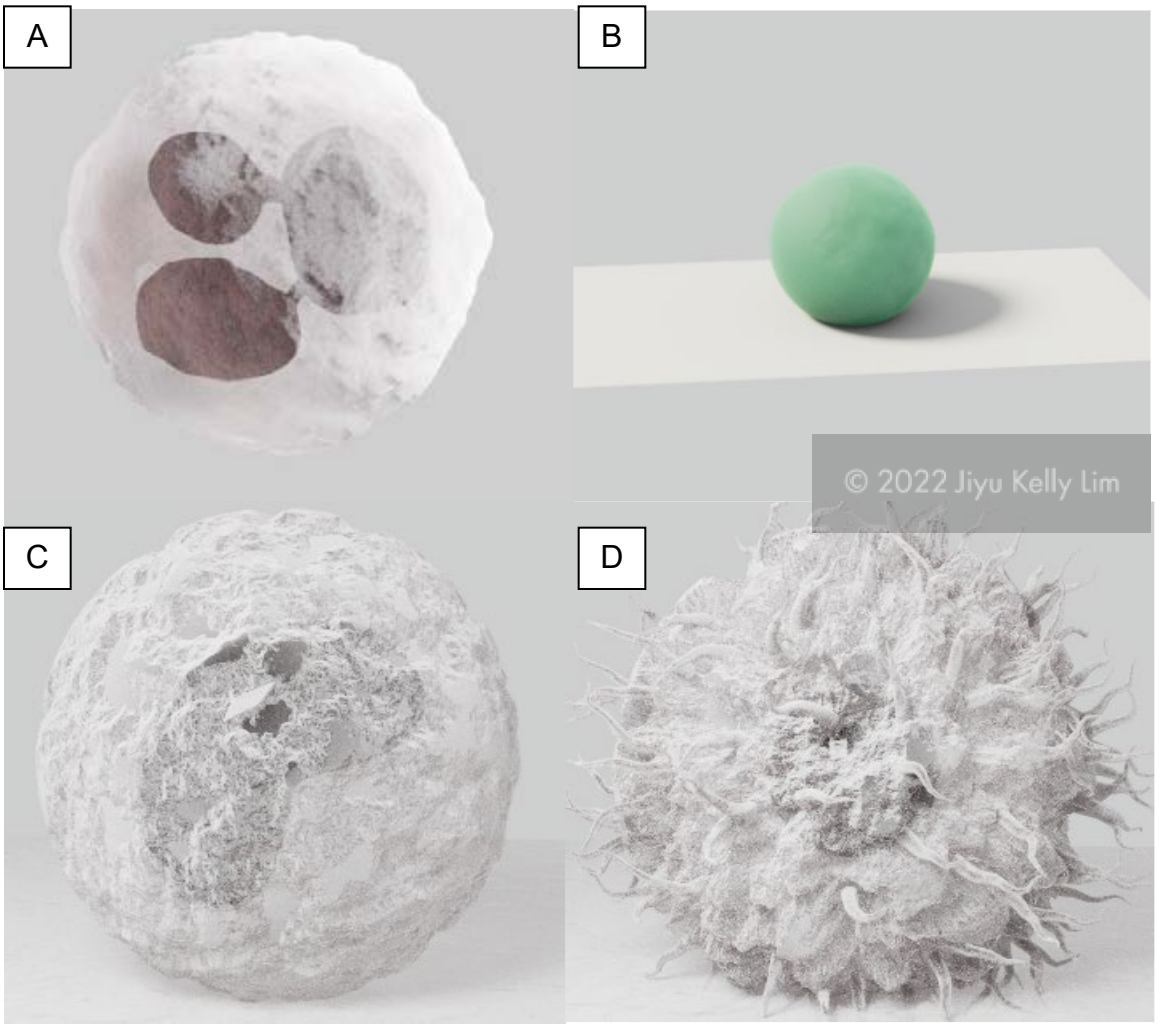


Figure 15. Leukocytes and bacteria. A) Neutrophil B) Streptococcus epidermidis C) Monocyte D) Macrophage

Leukocytes and Streptococcus epidermidis were modelled using spheres with **displacement** texture, and **hair** material when needed. Characteristic nuclei were built using spheres and the **Magnet** tool, **Volume Builder** and **Volume Mesher**.

iv. Lipid Bilayer

For heterogenicity, three lipid models of slightly different morphologies were researched and imported from the University of Calgary website: https://wcm.ucalgary.ca/tieman.

The molecules were optimized then placed within mograph **Cloner**, with Mode set to Object. A plane with a **Displacer** was created and selected as the Object. For the lower lipid layer, the Cloner was duplicated and rotated 180 degrees on the P axis. **Random** effector was used to add a natural look to the model.

To reduce rendering time, low poly models were created using **Polygon Reduction** and utilized for the lipid molecules far from the camera.

Figure 16. Close-up view of the lipid bilayer. A flat plane with a **Displacer** was used for **Cloner Object**.

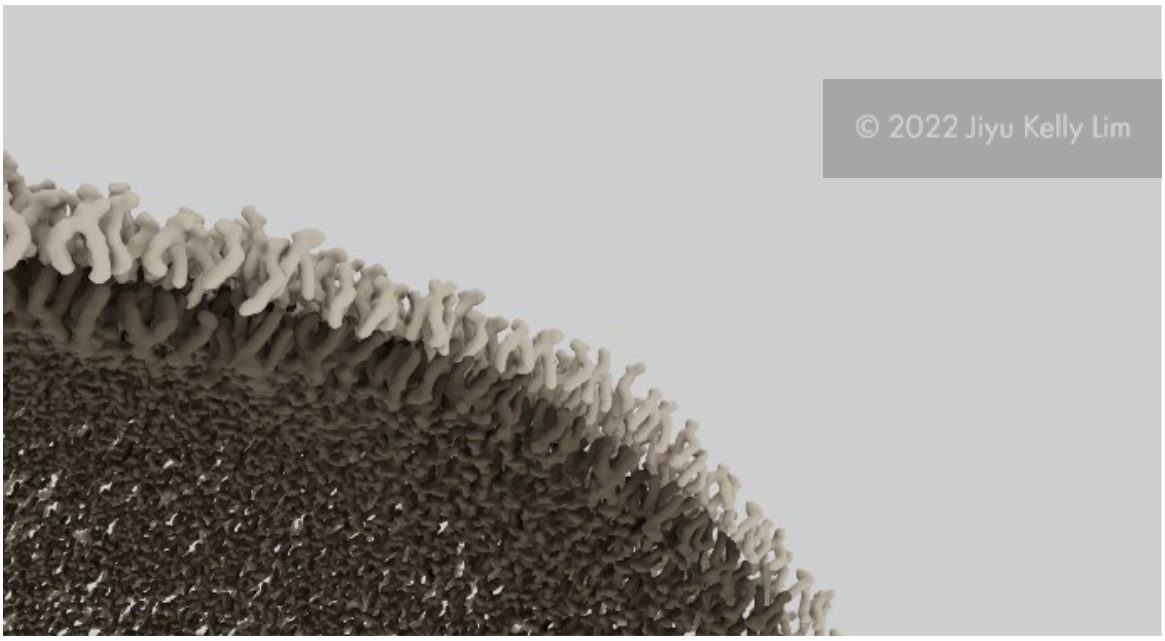


Figure 17. Distant view of the lipid bilayer. A bent plane was used for the **Cloner Object** to mimic cell shape.

v. Cell Protrusions

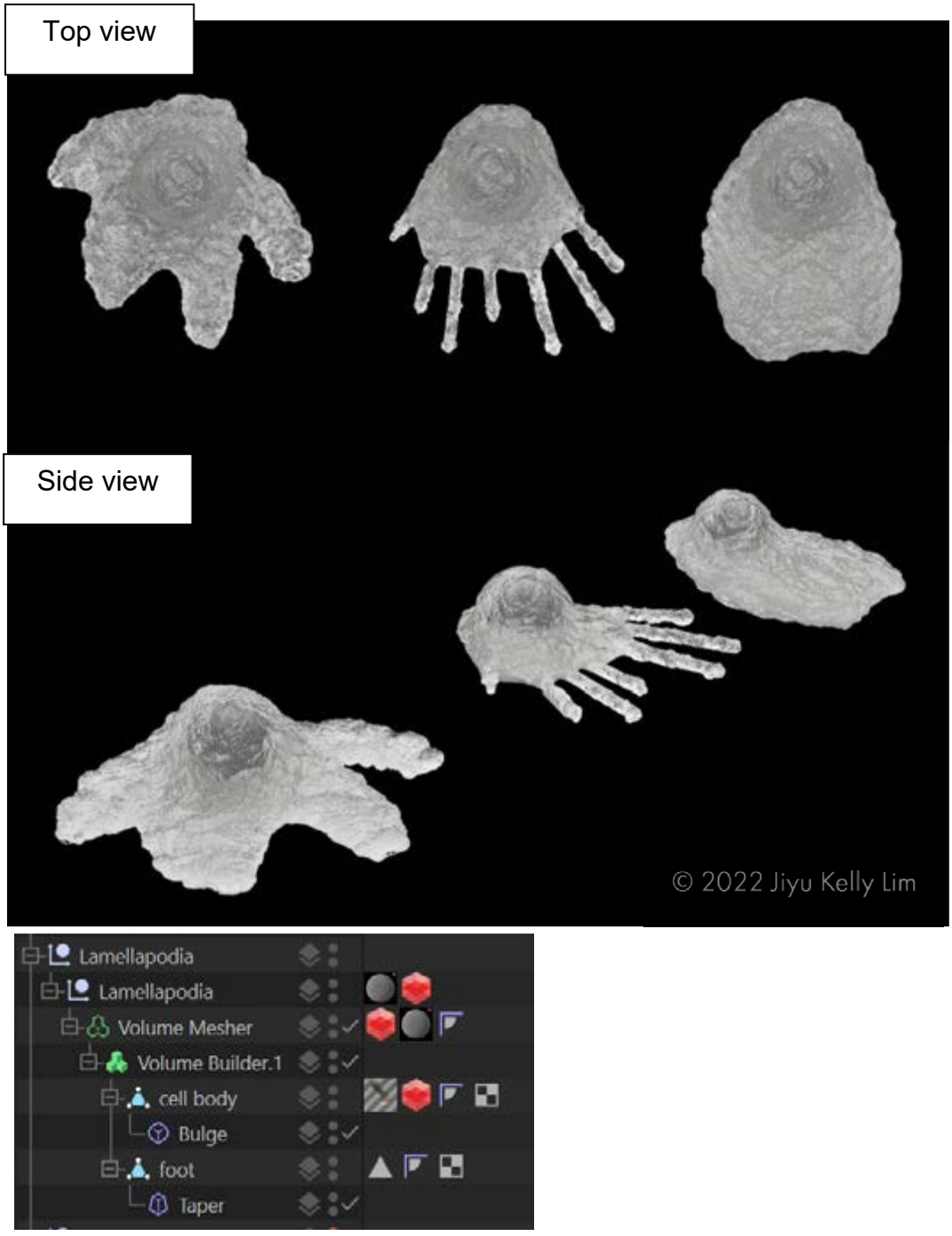


Figure 18. Different types of cell protrusions: pseudopodia, filopodia, and lamellipodia (from left to right). Object manger demonstratse how lamellipodia was created.

To create three types of distinctive cell protrusions, the cell body and the foot process were generated separately. The cell body was created from a sphere in **Bulge** deformer, which was shared between all three models.

In lamellipodia, the foot process was created from a flattened sphere in **Taper** deformer. In pseudopodia and filopodia, the foot process used in lamellipodia was scaled down appropriately and multiple copies made.

Finally, the cell body and the foot process were placed within **Volume Builder** and **Volume Mesher**.

vi. Cytoskeleton and Signaling Molecules

	Average Length (nm)	Average Diameter / Thickness (nm)
Lipid Bilayer	-	4 – 10
Actin Filament	36 - 151	5.4
Myosin II Monomer	186	5.6
Myosin II Dimer	200	13
Ras	-	2
PIP3	-	0.04
PTEN	-	8
PIP2	-	0.04

Table 1. Average lengths of diameters of key molecules.

	Ratio
PTEN	200
Ras	50
PIP3	1
PIP2	1

Table 2. Size ratio of key signaling molecules.

Actin

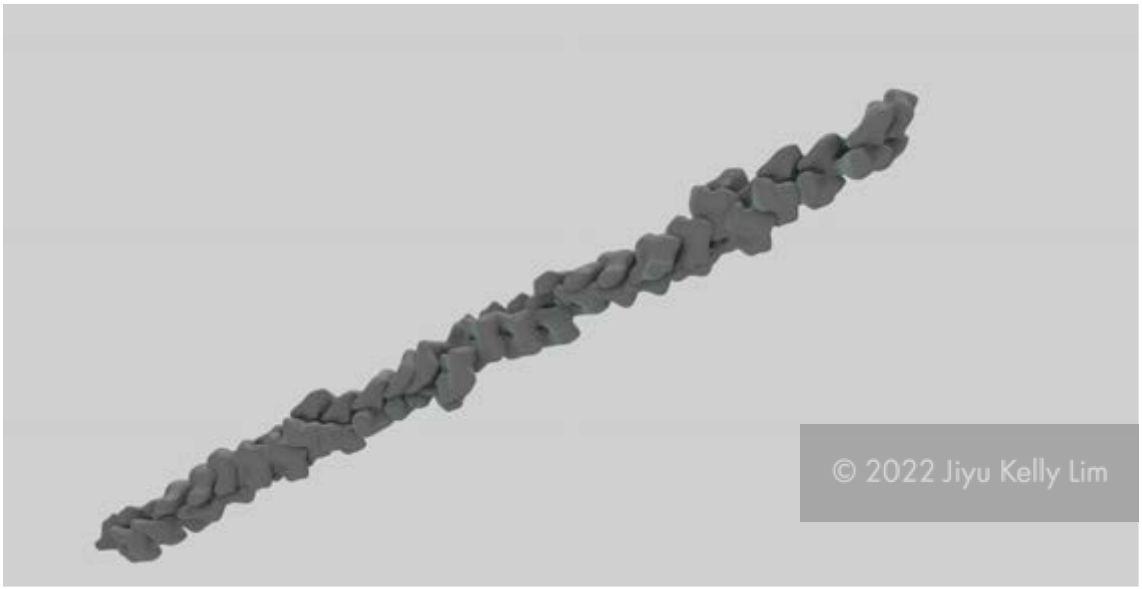


Figure 19. Actin polymer model.

The actin polymer in the Protein Data Bank (PDB) file 3G37 from www.rcsb.org was isolated into individual segments by using the command “split chain” then saved as a VDML file and imported into C4D. individual segment was placed in mograph **Cloner** to create a polymer.

Myosin II

Figure 20. Myosin II model. *Text not intended to be read.*

Myosin's head (5I4E) and tail (1D7M) were imported separately from www.rcsb.org into Cinema4D using the ePMV plug-in and welded together in Zbrush. The structure was also simplified into a low poly model in Zbrush using **ZRemesher**.

Signaling Molecules

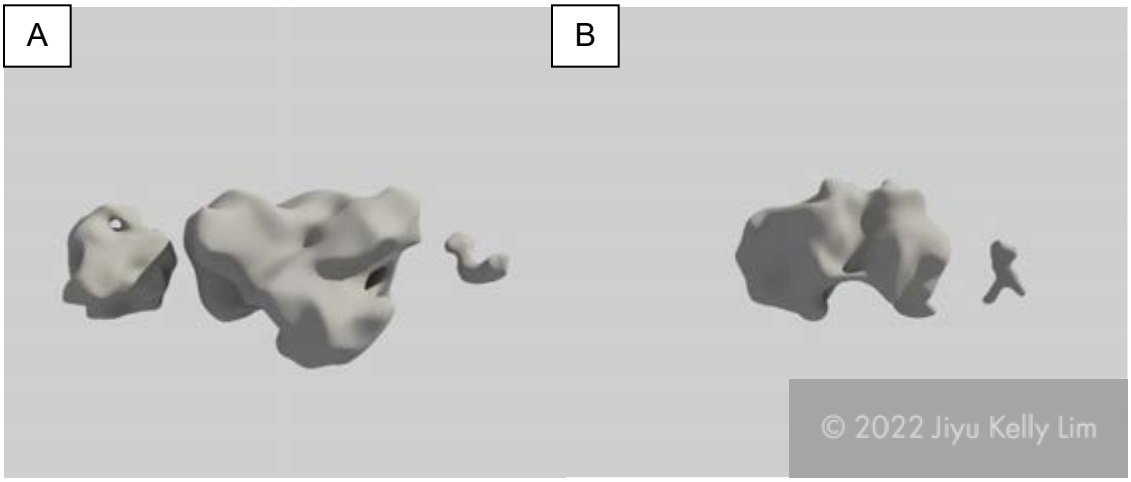


Figure 21. Signaling molecules models. A) Ras protein, PI3K, PIP3, from left to right B) PTEN and PIP2, from left to right

Each molecular structure (Ras: 5P21; PI3K: 5JHB; PIP3: 4RWV; PTEN: 1D5R; PIP2: 6CS9) was imported from www.rcsb.org to Cinema4D using the ePMV plug-in. Low poly models were also created in ZBrush using **ZRemesher**.

vii. Embryo

Three distinctive gestational ages were chosen: 7 weeks, 20 weeks, and full-term.

The transition from the first to second gestational ages demonstrates the formation of digits by apoptosis in the apical ectodermal ridge, as well as development of external ears and regression of the f tail.

The transition from the second to third gestational age suggests the formation of a full-term fetus with features that the viewer will recognize as a “baby”. Continued refinement of the external ears and digits are apparent. The stark morphological transition from the first stage to the third stage will emphasize the pivotal role of cell migration in embryogenesis.

All stages were modelled in ZBrush then imported to Cinema4D. Refences are based on textbooks: Langman’s Medical Embryology (12th Edition), Inderbir Singh’s Human Embryology (11th Edition), and Larsen's Human Embryology (6th Edition).

Figure 22. ZSpheres were used to build the basic structure of the model.

ZSphere was selected from 3D mesh and Click and Drag used to adjust size of the first sphere. Holding shift key snaps its alignment. Edit was clicked and Draw mode activated to create additional spheres to the first sphere. Symmetry Mode was activated throughout the process

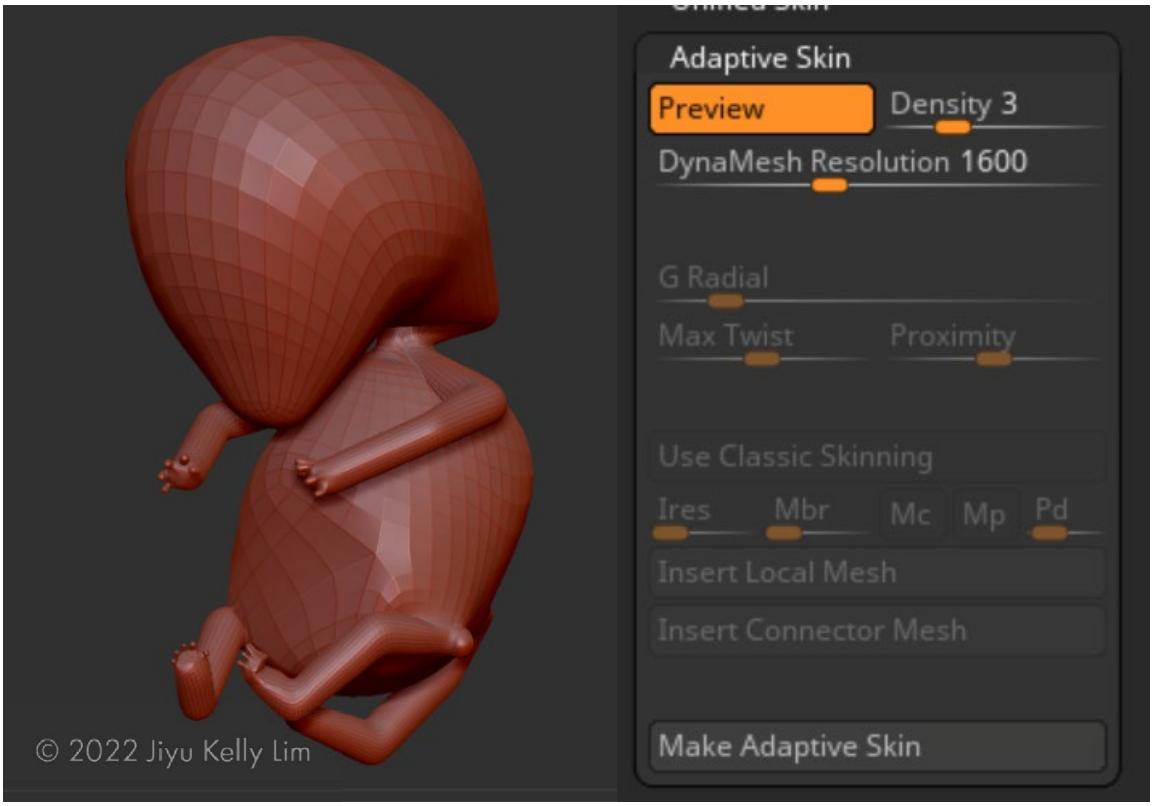


Figure 23. Model after Adaptive Skin has been applied.

Adaptive Skin was applied. **Preview** was clicked first to check the appearance. When satisfied **make Adaptive Skin** was clicked to make the skin into an editable mesh. **DynaMesh** with a Resolution of 1600 and Density of 3 was used for this model.

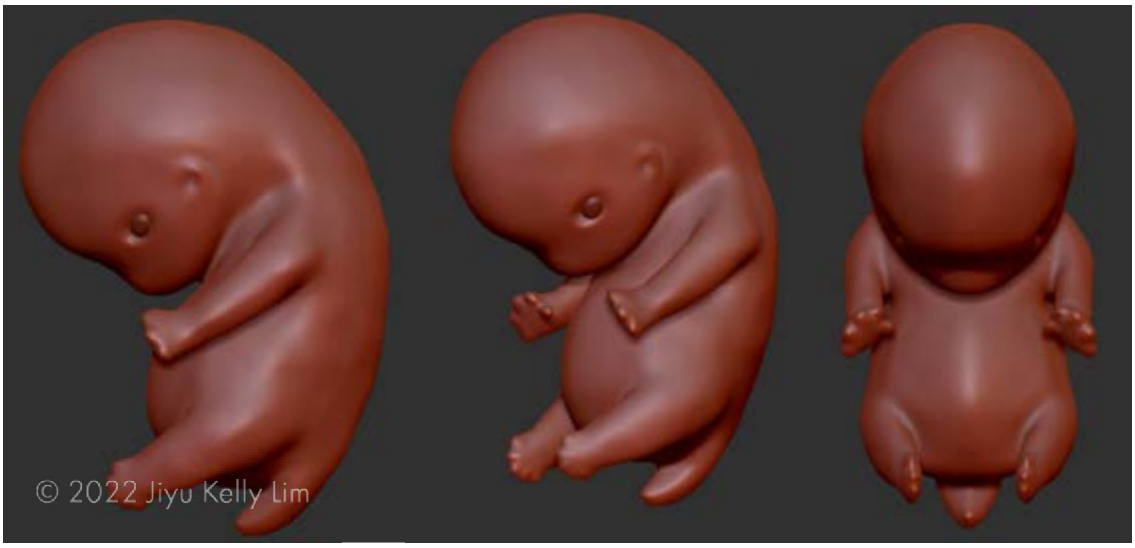


Figure 24: Embryo at 7 weeks. Limbs buds, fan-like digits and a tail are characteristic. Hind limb development lags behind forelimb development by a couple of days – hence their lengths are depicted to be shorter than the forelimbs.

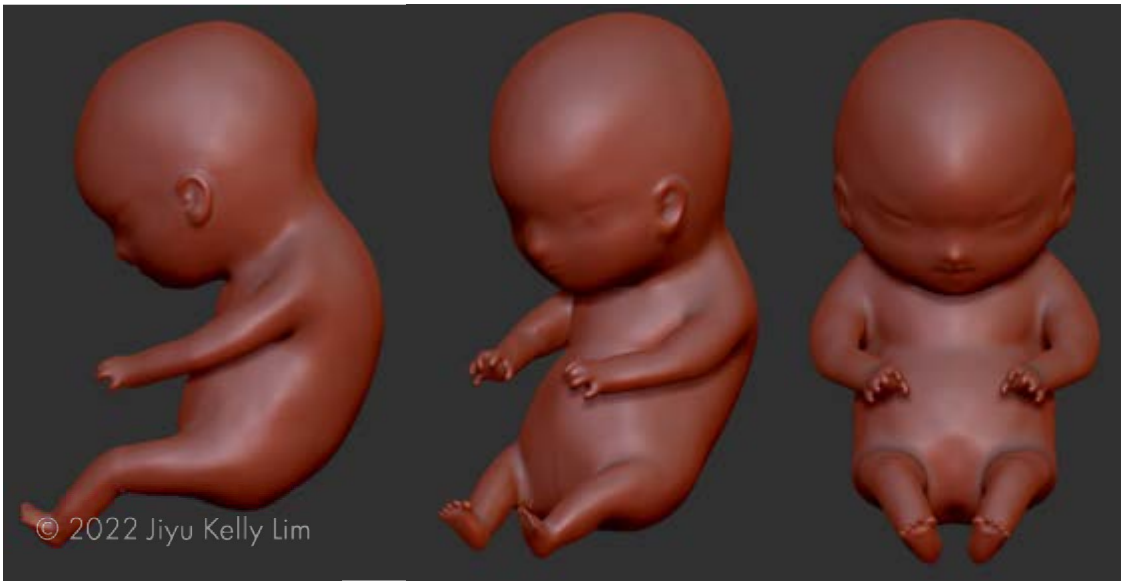


Figure 25. Fetus at 20 weeks. Limbs have elongated and undergone 90-degree rotation, upper limbs laterally and lower limbs medially. Eyes are covered by eyelids, and external ears have started to form.

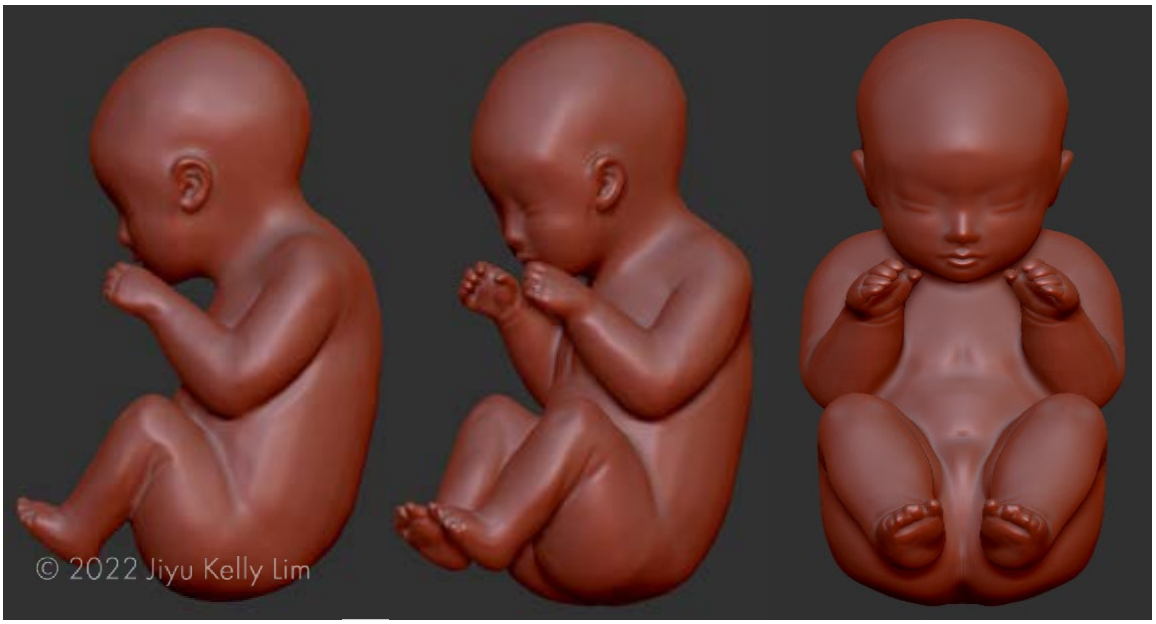


Figure 68. Full-term fetus. Limbs have grown to the appropriate ratio and rotation with fully refined digits and external ears.

Optimizing and Importing Models into Cinema4D

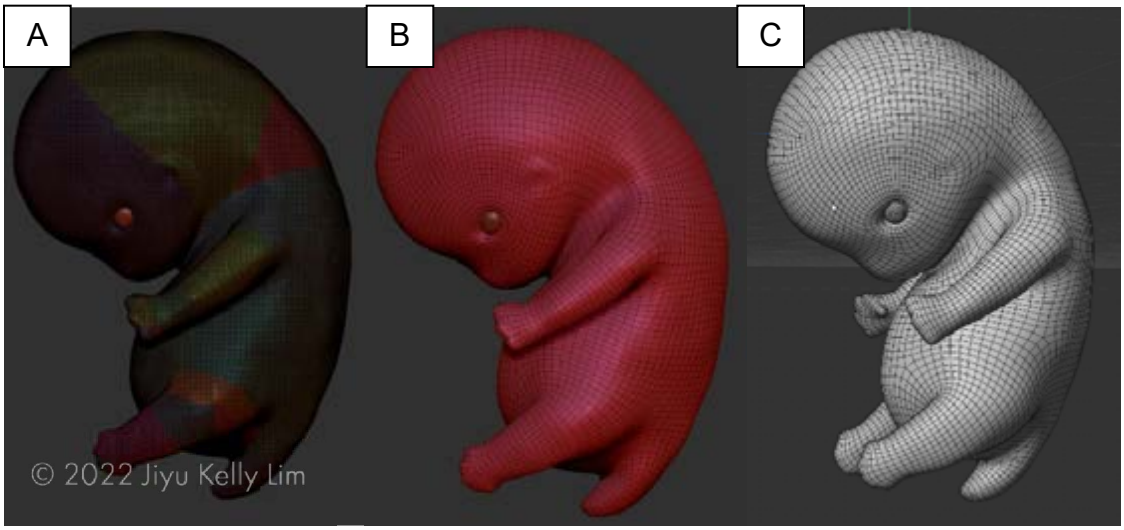


Figure 27. Model optimization. A) Each part of anatomy is assigned to a different polygroup for optimal modelling workflow. B) Model after **ZRemersher** has been applied. C) Model after it has been imported into Cinema4D.

The embryo was first modelled in high subdivision settings then polygon count was reduced by **ZRemesher.** The lowpoly model was imported to Cinema4D by clicking on **GoZ** in ZBrush then **Extensions** > **GoZBrush** > **GoZImporter** in Cinema4D.

Rendering

Redshift was used for textures, lighting, and camera movements. It is a biased GPU render engine that has fine control on render settings with a fast rendering speed and an interactive real-time render view.

The blackberry scene is presented as an example of rendering.

Figure 28. Blackberry scene before rendering.

i. Texture

Figure 29. Blackberry scene with textures added.

A variety of material application methods was used. The texture used to represent a half-ripe blackberry is presented as an example (Figure C Below). To reproduce its characteristic color where both red and green can be seen, two separate materials were created and mixed by **Material Blender**. Crimson material was used as a base color. To create a map that instructs where the second color (green) will apprear, **Curvature** node was created and placed in Blend Color 1.

Both materials had roughness values of >0 and reflection value of <1 to re-create the rough, matte texture of a half-ripe blackberry.

Figure 30. Breakdown of materials used for a half-ripe blackberry.

ii. Lighting

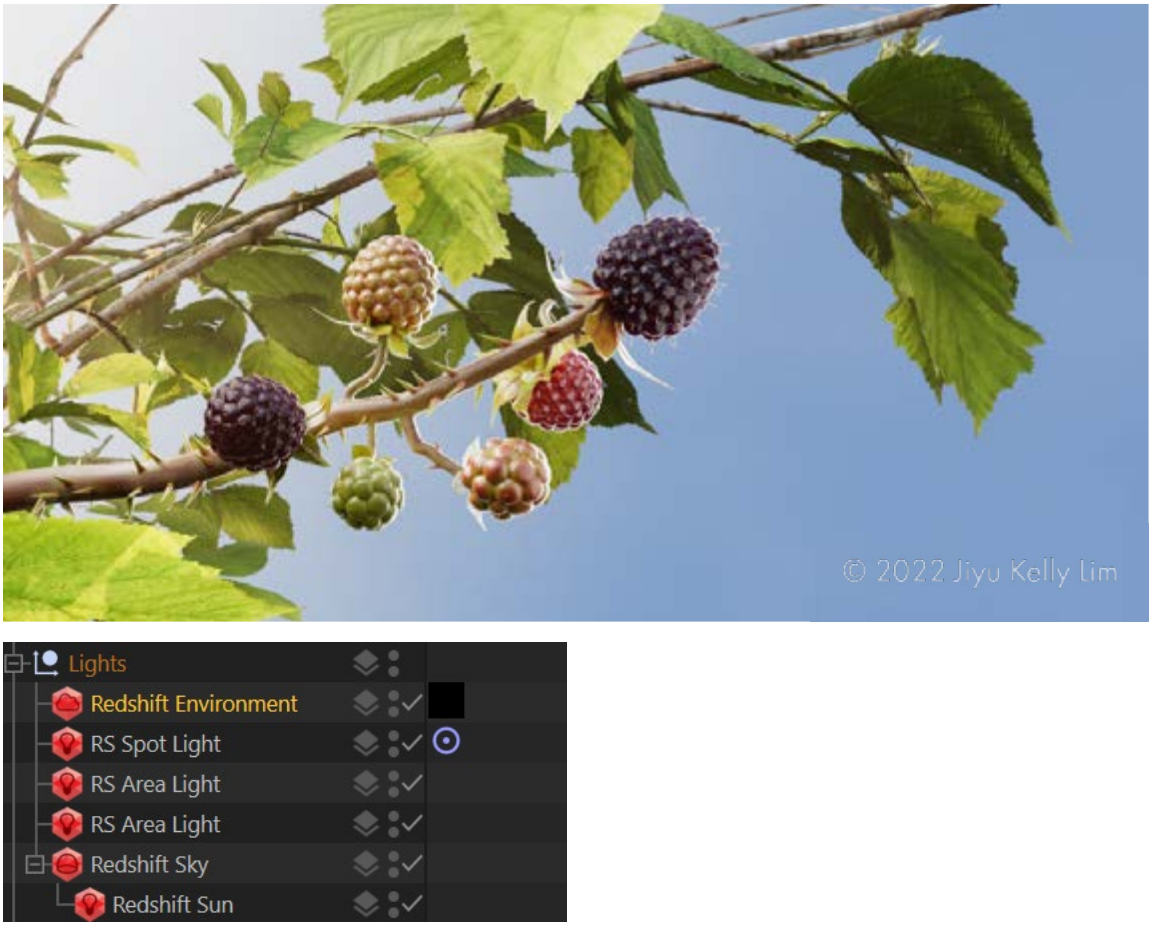


Figure 31. Blackberry scene after lights have been added.

Different lights were used to reproduce the natural light on a sunny day. First **Sky with Redshift Sun** was placed then two **Area Lights** were used as Back and Fill Lights. A **Spot Light** was used as the Key Light.

Finally, **Redshift Environment** was created so that the light rays hitting the branch could be visualized.

iii. Camera Set-up

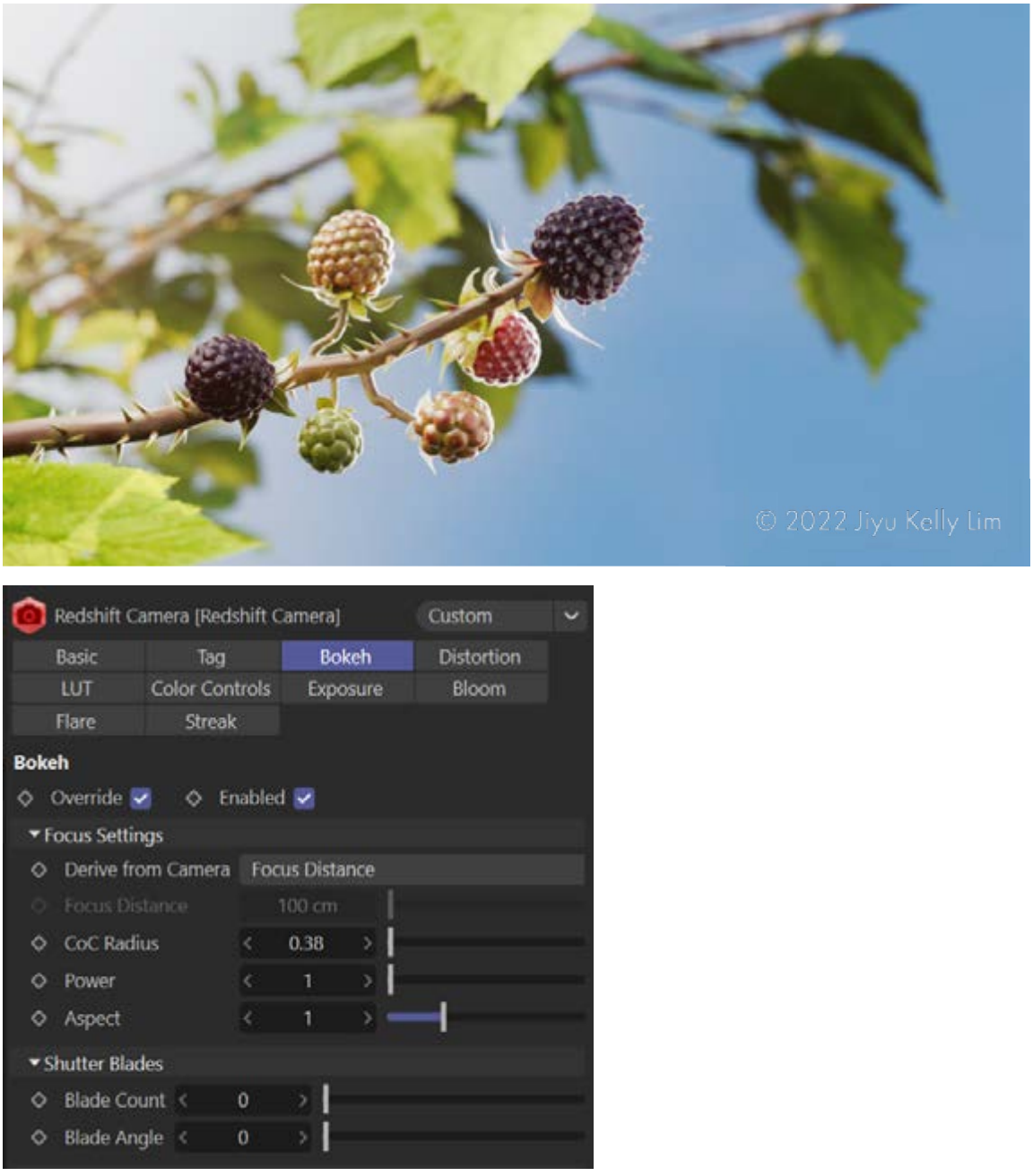


Figure 32. Blackberry scene after camera has been set up.

A Redshift Camera was placed with **Bokeh** enabled. **Focus Distance** was selected, and **CoC Radius** was set at 0.38 so that the distant assets would be blurred.

Post-Production Editing

Adobe After Effects was used for post-production editing, including text and 2D image animation, audio-to-video synchronization, color correction and special effects.

Results

Figure 33. 3D Animation still. A hand gets pricked by a thorn while trying to pick a blackberry.

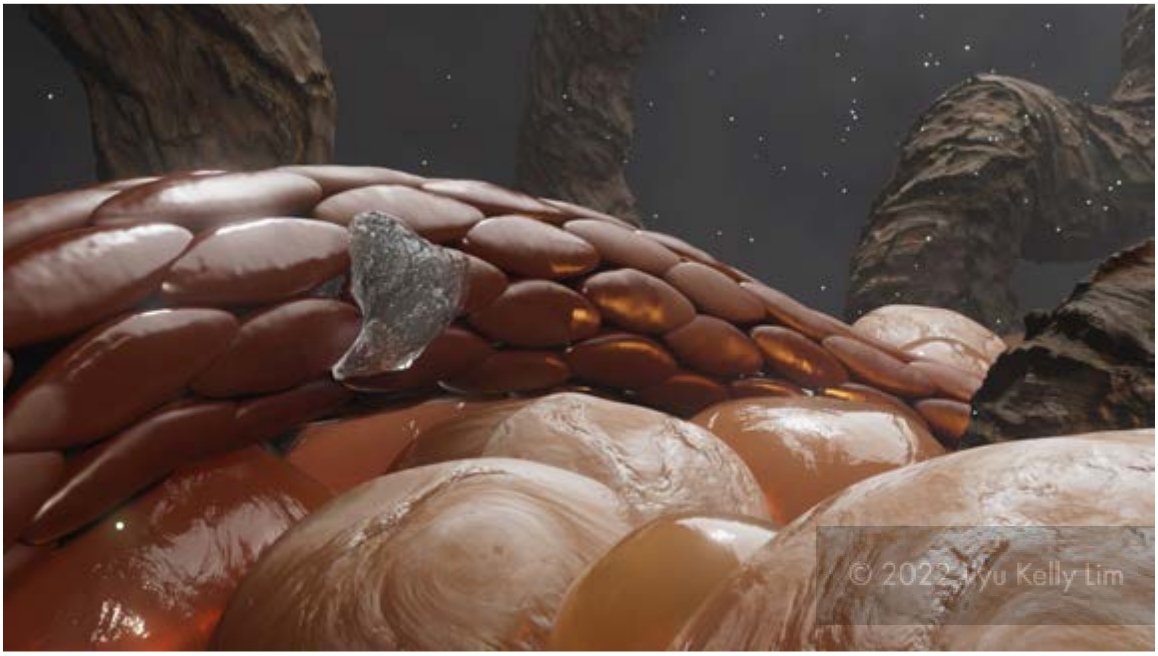


Figure 34. 3D Animation still. A neutrophil extravasates from a leaky blood vessel.

Figure 35. 3D Animation still. A neutrophil is migrating towards the site of injury.

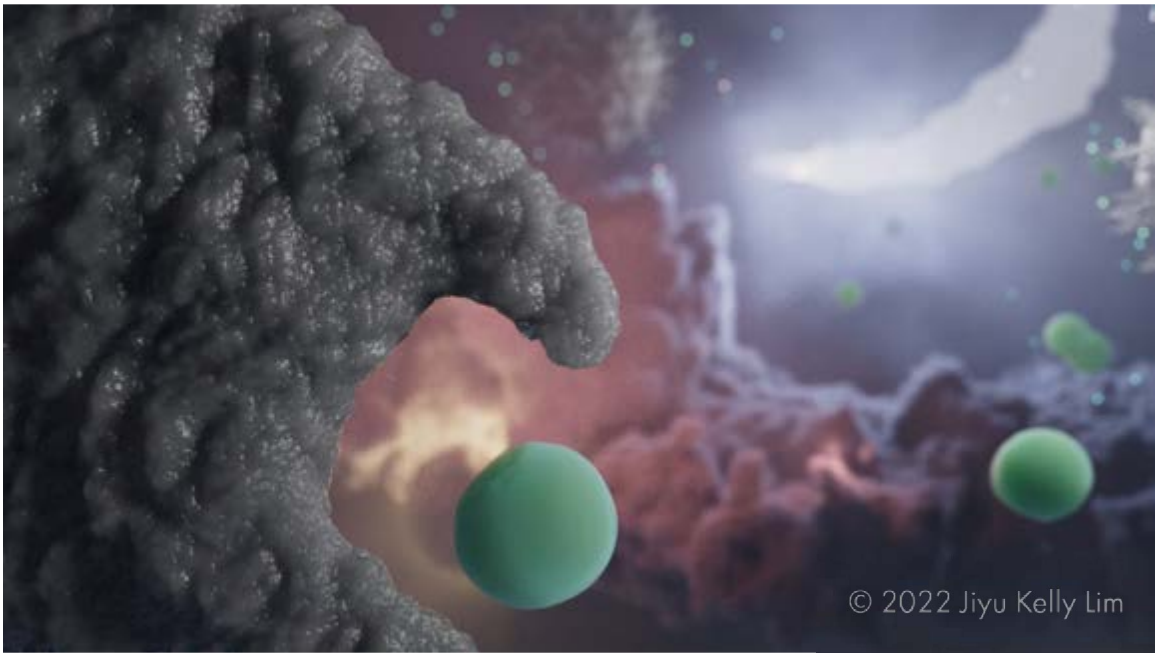


Figure 36. 3D Animation still. Streptococcus epidermidis is getting phagocytosed by a neutrophil.

Figure 37. 3D Animation still. Filopodia is depicted.

Figure 38. 3D Animation still. Pseudopodia is depicted.

Figure 39. 3D Animation still. Lamellopodia is depicted.

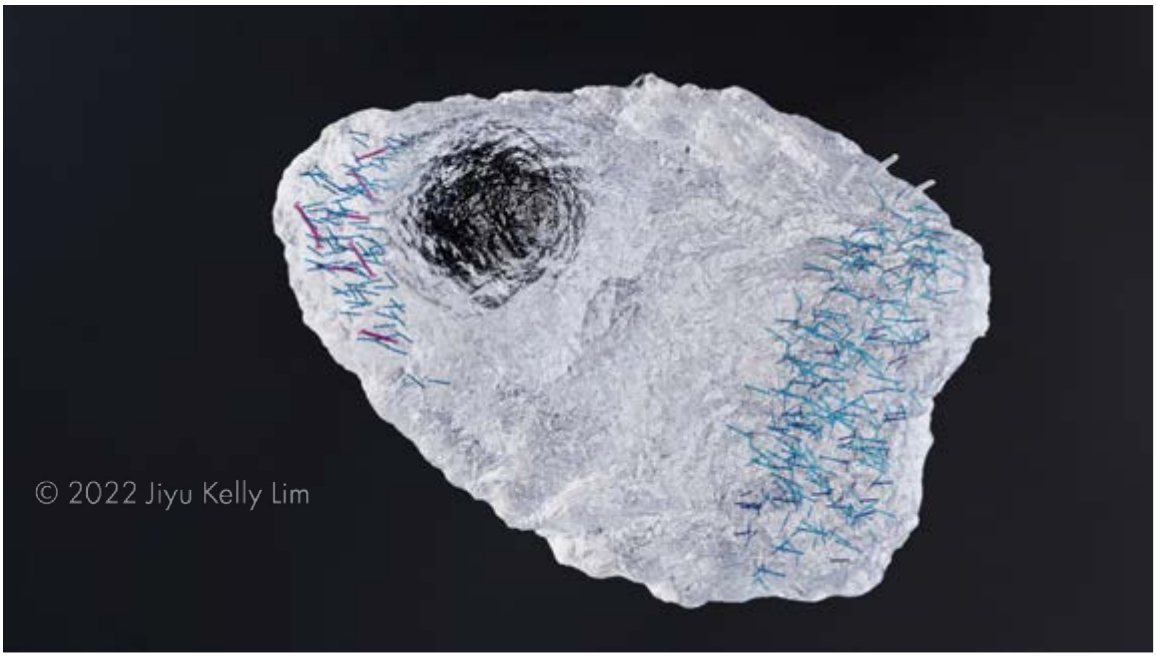


Figure 40. 3D Animation still. Cytoskeletal activity is depicted.

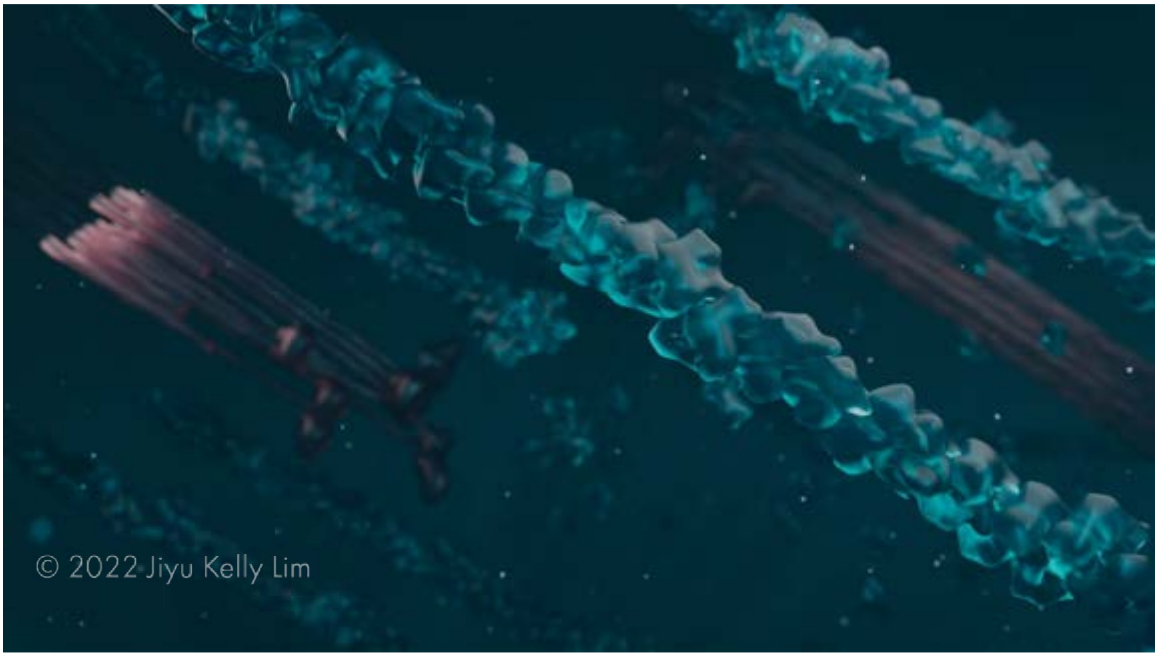


Figure 41. 3D Animation still. Cytoskeletal activity is zoomed in.

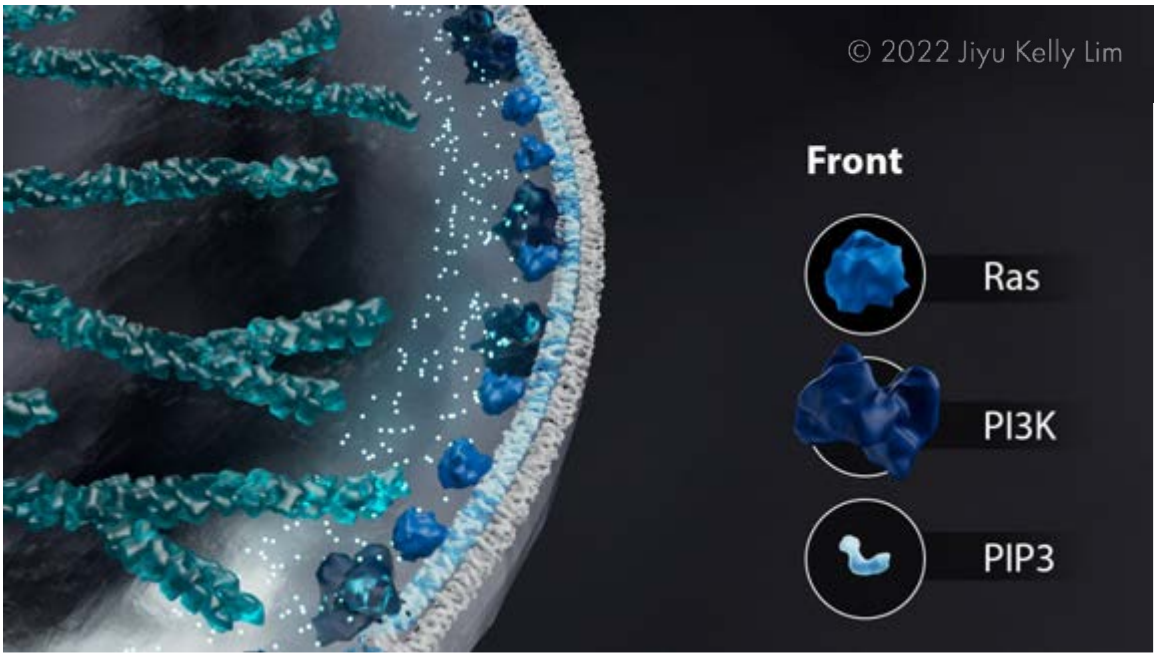


Figure 41. 3D Animation still. The interplay between STEN and cytoskeletal activities at the leading edge is depicted.

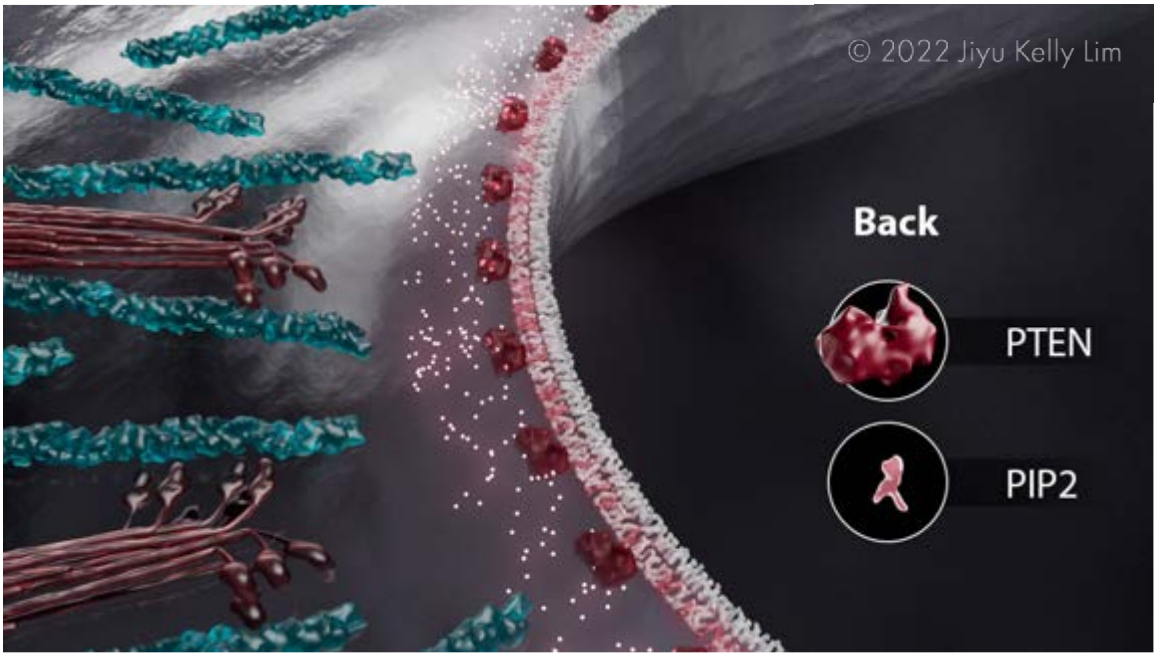


Figure 42. 3D Animation still. The interplay between STEN and cytoskeletal activities at the trailing edge is depicted.

Figure 43. 3D Animation still. Embryogenesis is depicted.

Figure 44. 3D Animation still. Nervous system wiring is depicted.

Figure 45. 3D Animation still. Immune regulation is depicted.

Figure 46. 3D Animation still. Metastasis is depicted.

Access to Assets

The animation resulting from this thesis can be viewed at https://cellbio.jhmi.edu/ and https://kellylimstudio.com/. The author can be reached through the Department of Art as Applied to Medicine at https://medicalart.johnshopkins.edu/.

Discussion

Project Objectives

The primary objective of this project was to create a 3D animation to assist viewers in understanding the mechanism and significance of Signal Transduction Excitable System (STEN). The intended audience for the animation is undergraduate and graduate biochemistry students and medical students who already have at least a basic understanding of cell migration. The project was based on both well-established scientific understanding of the topic as well as recent novel findings about the details of the mechanism and regulation of STEN.

The first portion of the animation is devoted to explaining the fundamentals of cell migration. Later portions of the animation cover signal transduction activities. The animation demonstrates how STEN interacts with cytoskeletal activity to determine the dimensions and locations of cellular protrusions. Finally, the animation shows cell activities where the interplay between STEN and cytoskeletal activity plays a pivotal role in key functions of the cell including migration, division, phagocytosis, and cancer metastasis.

A primary goal of the project was to cover all important aspects of signaling transduction activities while keeping the content succinct and the duration of the final animation short. Rendering was designed to be cinematic and visually appealing to captivate viewers with the beauty of the cell, and perhaps inspire them to pursue the study of cell biology or the field of medical illustration.

Snapshots of the 3D animation were rendered in schematic 2D diagrams so they may be used independently if desired.

The final 3D animation was 2 minutes 57 seconds in duration.

Challenges During Project

The most significant challenge encountered during the project was overcoming the unfamiliarity, complexity, and novelty of the topic. A number of meetings and a vast amount of research were required to make the animation comprehensive and accurate.

Refining and editing large amounts of information gathered during the research of the project to create a short, succinct script was also a significant challenge, particularly given the complexity and still incompletely understood nature of the topic.

Creating 3D assets posed another challenge. Rendering structures accurately in correct relative sizes while keeping polygon counts low required extensive research, calculations, careful modeling, and polygon optimization.

Future Directions

A Future direction of this project is to build additional multimedia tools to aid in better describing this complex topic, for example, an interactive website. to broaden accessibility and increase general interest. A well-designed interactive website would also further pique audience interest and captivate attention ultimately leading to better public understanding of the topic. Many studies have found interactive tools enhance viewers' learning by aiding long-term memory formation and increasing learning satisfaction (Ilhan et al. 2016, Abdulrahaman et al. 2020).

Topics that were only briefly mentioned in this animation, such as cytoskeletal activitiy and examples where the interplay between signal transduction plays a pivotal role in cellular processes may be explored further in supplemental animations or via an interactive website.

Appendix A: Script

Life is dynamic. Living cells are always changing and transforming themselves in one way or another. For instance, many cells are constantly changing their shapes by displaying a variety of protrusions that bring about cellular activities, such as migration, division, and phagocytosis.

Examples of these shape-altering protrusions include finger-like filopodia, broader pseudopodia, and sheet-like lamellipodia. What triggers these extensions and determines their features?

The mechanical forces that drive the protrusions outward from the cell body are mediated by combined actin polymerization and actomyosin-based contractions, referred to as “cytoskeletal activity”.

However, cytoskeletal activity alone produces only transient, small extensions, or “puncta” or “ruffles”, which cannot effectively move or reshape the cell.

The extensions attain their dynamic shapes and locations through the action of a network of components, including small GTPase proteins and phosphoinositide lipids, collectively referred to as Signal Transduction.

Upon activation, the network components quickly organize into “front” or “back” regions on the inner surface of the membrane. Front activities such as Ras, PI 3-kinase, and PIP3 locate at the tip of protrusions and promote actin polymerization, while back molecules such PTEN and PIP2 leave the “active” regions and accumulate at the trailing edge of the cell, leading to actomyosin-based contractions.

The protrusions expand as the region's network "fires" sequentially, sweeping across the inner face of the membrane like a laterally moving stadium wave. Increasing or decreasing signal transduction activity can elevate or decrease the speed and range of wave propagation, converting pseudopodia into wider lamellipodia, or narrower filopodia.

The spontaneous "firing" of the signal transduction network causes the cell to move in random directions. But external chemical, mechanical, or electrical signals influence this spontaneous firing to guide the cell and direct its migration and behavior.

A highly orchestrated control of these dynamic changes in cell shape and motility not only guide immune cells to a pricked finger but bring form to the embryo, wire the nervous system, and have clinical implications in wound healing, tissue regeneration, and cancer metastasis.

Appendix B: Storyboard

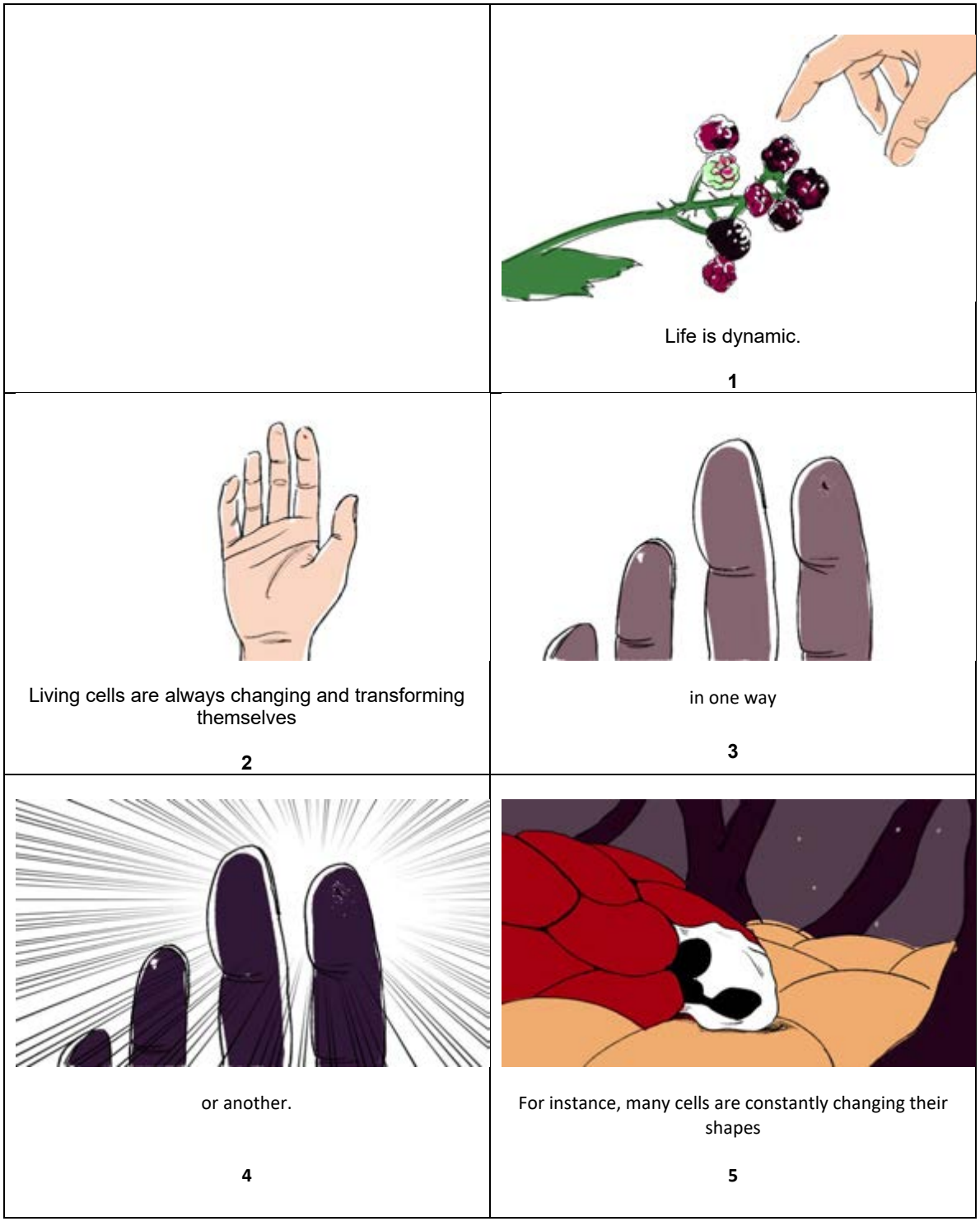


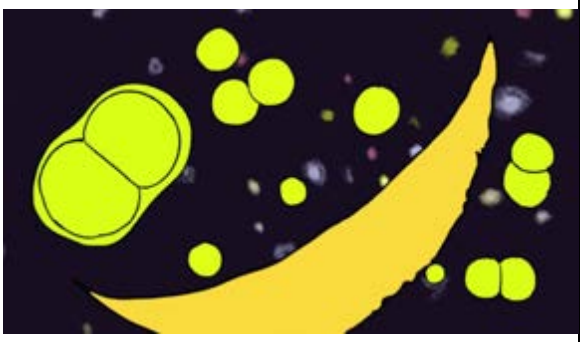
Figure 47. Storyboard, page 1.

by displaying a variety of protrusions that bring about important cellular activities,

6

such as migration,

7



division,

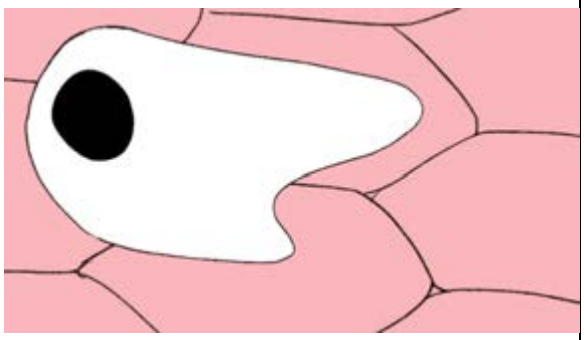
8

and phagocytosis.

9

Examples of these shape-altering protrusions include finger-like filopodia,

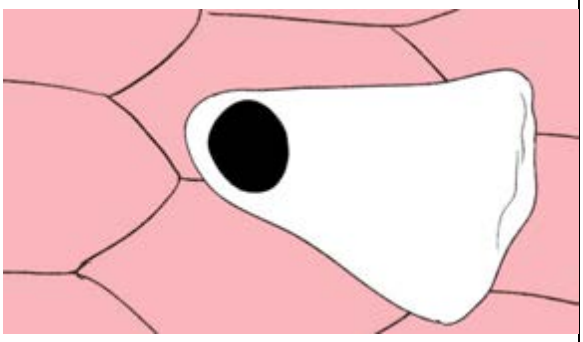
10



broader pseudopodia,

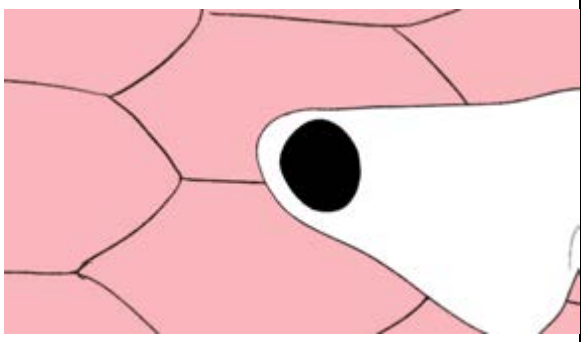
11

Figure 48. Storyboard, page 2.



and sheet-like lamellipodia.

12



What triggers these extensions and determines their features?

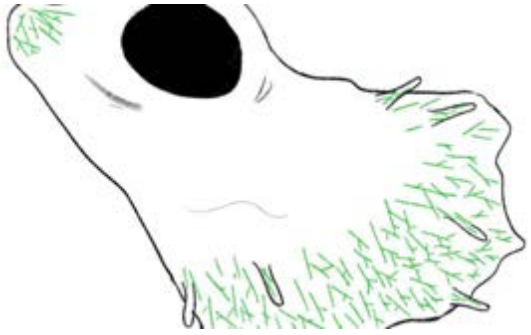
13

The mechanical forces that drive the protrusions

14

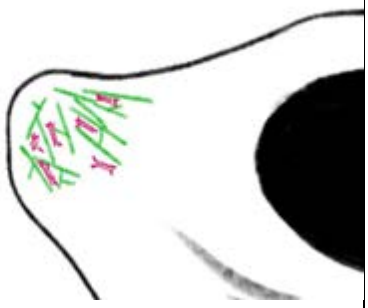
outward from the cell body are mediated by

15



combined actin polymerization

16



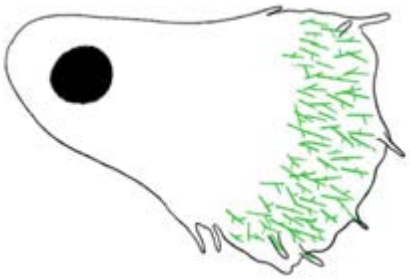
and actomyosin-based contractions,

17

Figure 49. Storyboard, page 3.

referred to as “cytoskeletal activity”.

18



However, cytoskeletal activity alone produces

19

only transient, small extensions, or “puncta”

20

or “ruffles”, which cannot effectively move or reshape the cell.

21

The extensions attain their dynamic shapes

22

and locations

23

Figure 50. Storyboard, page 4.

through the action of a network of components, **24**	including small GTPase proteins and phosphoinositide lipids, collectively referred to as Signal Transduction. **25**
Upon activation, **26**	the network components quickly organize into “front” **27**
or “back” regions on the inner surface of the membrane. **28**	Front activities such as Ras, PI 3-kinase, and PIP3 locate at the tip of protrusions **29**

Figure 51. Storyboard, page 5.

and promote actin polymerization, **30**	while back molecules such PTEN and PIP2 PIP2 leave the “active” regions and accumulate at the trailing edge of the cell, **31**
leading to actomyosin-based contractions. **32**	The protrusions expand as region’s network activation “fires” sequentially, **33**
sweeping across the inner face of the membrane like a laterally moving stadium wave. **34**	Increasing or decreasing signal transduction activity **35**

Figure 52. Storyboard, page 6.

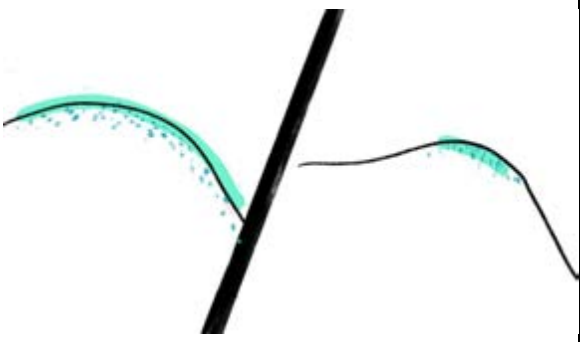
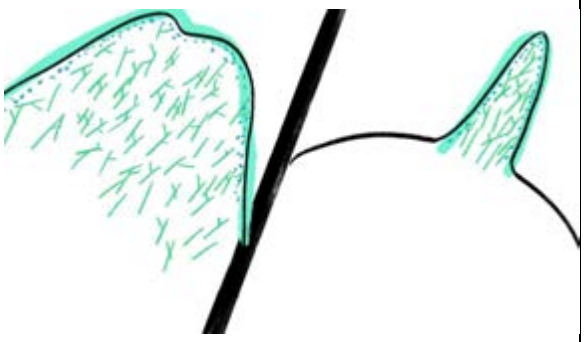
 can elevate, or decrease the speed and range of wave propagation, **36**	 converting pseudopodia into wider lamellipodia, or narrower filopodia. **37**
The spontaneous “firing” of the signal transduction network causes the cell to move in random directions. **38**	But external chemical, **39**
mechanical, **40**	or electrical signals **41**

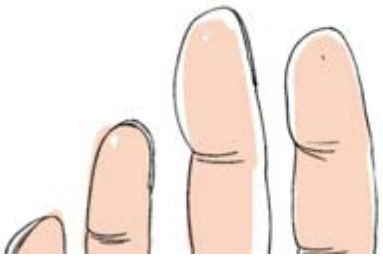
Figure 53. Storyboard, page 7.

influence this spontaneous firing to guide the cell and direct its migration and behavior. **42**	A highly orchestrated control of these dynamic changes **43**
in cell shape and motility **44**	 not only guide immune cells to a pricked finger **45**
 but bring form to the embryo, **46**	 wire the nervous system, **47**

Figure 54. Storyboard, page 8.

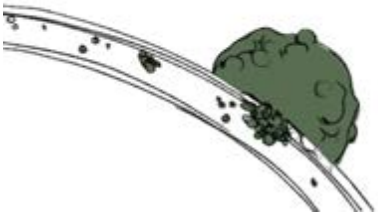
and have clinical implications in wound healing,

48



tissue regeneration,

49



and cancer metastasis.

50

Figure 55. Storyboard, page 9.

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Vita

Jiyu Kelly Lim was born in Seoul, Korea and spent her childhood in Canada and the UK. Kelly has always been fascinated by both art and science. Unable to focus on one, she initially pursued a career in medicine. Following her graduation in 2017 with a medical degree from Imperial College London, she worked as a junior doctor for two years in various specialties, ranging from Emergency Medicine to Geriatric Psychiatry. Experience with diverse patients made her realize the importance of effective medical communication, and she began to seek a career path where she could maximize not only patients', but general public's understanding of medicine.

In August 2020, Kelly began her studies in the Medical and Biological Illustration graduate program in the Department of Art as Applied to Medicine at Johns Hopkins University School of Medicine. During her time at Johns Hopkins, Kelly received William P. Didusch Scholarship, W. B. Saunders Scholarship, and Gwynne M. Gloege Scholarship.

At the 2021 Annual Association of Medical Illustration conference, Kelly received an Award of Excellence in the student category for a neuroanatomy piece titled "Relative Positions of Internal Capsule and Corona Radiata to Basal Ganglia and Cortical Regions". She is currently a candidate to receive a Master of Arts in Medical and Biological Illustration in May 2022.

After graduation, Kelly hopes combine her artistic talent and skills with her medical background to translate the complex world of science and medicine into simple, beautiful, and compelling images.