INTRAARTERIAL TRANSPLANTATION OF GLIAL PROGENITORS TO REGENERATE BRAIN DAMAGE FOLLOWING RADIATION THERAPY OF BRAIN TUMOR:
AN ANIMATION FOR LAY AUDIENCE AND SCIENTISTS

by
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ABSTRACT

Each year, approximately 100,000 patients in the United States develop radiation-induced brain injury as a consequence of receiving radiation therapy treatment for brain tumors. Myelin-producing oligodendrocytes are affected by radiation, can result in demyelination leading to the deterioration of axonal function, and over time may cause irreversible damage of neurons. Impaired neurological function leads to problems with feeling, thinking, and moving, all having substantial negative effects on a patient’s quality of life.

These problems can potentially be solved by regenerating damaged myelin sheaths using glial restricted progenitors (GRP’s). GRP’s are precursors of oligodendrocytes, which grow into mature oligodendrocytes able to remyeliinate radiation damaged areas. GRP’s hold promise for a new approach to treatment.

In Dr. Piotr Walczak’s lab at the Institute for Cell Engineering (ICE), at The Johns Hopkins University School of Medicine, a pre-clinical rodent study is being used to develop methods for safe and efficient cell delivery to brain lesions. GRP’s are modified with iron oxide nanoparticles making cells visible under MRI. An adhesion molecule, Very Late Antigen-4 (VLA-4), is used to control cell binding to blood vessels within the damaged region of the brain. Cell delivery methodology is improved using MRI to monitor the procedure.

No effective product exists that communicates this important and promising research in applying regenerative medicine solutions to oncology for either a lay audience or scientists. Multimedia enhances the quality of learning by using both the auditory and visual channels. Animations accompanied by narration are more effective learning tools than silent videos. Multimedia incorporating both 2D and 3D allows an audience to effectively learn complex information in a short time. This approach is appropriate for conveying information about transplant of GRP’s.
For this project, a narration, storyboard, and an animatic, were produced in preparation for developing an animation. A variety of softwares were used to create the animation. The 4 ½ minute narrated animation will show the principles of the concept for regeneration of the central nervous system. The animation will be made available on the webpage of Dr. Walczak’s laboratory and on the website of the Institute for Cell Engineering. The intent is to increase interest in this interdisciplinary approach, and to encourage funding for future research, leading to safer and more effective therapies of brain tumors.

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INTRODUCTION

Background: Radiation-Induced Brain Injury

When using radiation to destroy a targeted brain tumor, the brain tissue surrounding the tumor can also be damaged by radiation. In the U.S. each year, approximately 100,000 patients develop radiation-induced brain injury from brain tumor radiation therapy (Greene-Schloesser & Robbins, 2012). The injury has substantial negative effects on patients’ quality of life, because it can induce problems such as difficulty in thinking clearly, memory loss, personality change (Cancer Research UK, 2015), attention deficit (Greene-Schloesser & Robbins, 2012), and movement control (Harrisingh et al., 2015). These issues are particularly important in pediatric patients (Butler & Mulhern, 2005). These issues are of high concern in pediatric patients with respect to their development and schooling.

Stem cell therapy of neurological diseases

In stem cell therapy of neurological diseases, replacing lost neurons is very difficult (Li et al., 2012). A promising strategy for therapy of radiation-induced brain injury is replacing lost glial cells.

Demyelination

The central nervous system, or CNS is made up of the brain and spinal cord. The CNS is composed of grey matter and white matter. The grey matter contains most of the neuron cell bodies, and the white matter contains myelinated axons. In the CNS, axons are wrapped by myelin sheaths. The myelin protects the axon, and insulates the electrical impulses that allow neurons to transmit impulses quickly and efficiently. Myelin sheaths are composed of multiple concentric layers of plasma membrane produced by oligodendrocytes.
In radiation-induced brain injury, there is commonly loss of white matter via demyelination. This pathological process, and the related functional deficits, are progressive and become irreversible (Greene-Schloesser & Robbins, 2012). In the early stages of damage, glial cells including oligodendrocytes are affected. Myelin damage may lead to deterioration of the axon and the neuron itself, and in turn to problems with feeling, thinking, and moving. If demyelination persists, there may be irreversible damage to neurons and permanent loss of neurological function.

**Pre-Clinical Study: GRP Transplantation, Challenges and Solutions**

Oligodendrocytes are derived from immature cells called glial restricted progenitors (GRPs). GRPs efficiently generate myelin-forming oligodendrocytes in vivo (Goldman et al., 2012). The functions of oligodendrocytes have been studied in animal models such as rats and mice.

However, therapy based on transplantation of cells such as oligodendrocytes is challenging. The key issue is developing efficient cell delivery methods. Typically, cells are injected into the brain parenchyma via needles, resulting in distribution into only a very small narrow area. To solve this problem by allowing wider cell distribution, the intraarterial approach has been proposed. However, this approach bears several risks factors (Janowski et al., 2013).

The first risk is that the GRPs might not adhere at the targeted site, but pass through and become trapped in the wrong places in the body, such as the lungs. A second risk is that excessive adhesion and overabundance of the GRPs being delivered, could cause them to accumulate at a site in the blood vessels, blocking blood flow and leading to microembolism and stroke. To address these issues, there is a need to modify the cells, and monitor the injection procedure with imaging (Walczak, 2015; personal communication).
Introduction

In Dr. Piotr Walczak’s lab at the Johns Hopkins University Institute for Cell Engineering, GRPs are labeled with superparamagnetic iron oxide (SPIO) nanoparticles, making the cells visible under MRI, thus enabling an observer to monitor their location and actions. In addition, the GRPs are treated with an adhesion molecule, Very Late Antigen-4 (VLA-4), so that they are adherent to areas of the damaged brain. VLA-4 is a dimer that signals leukocytes to bind to Vascular Cell Adhesion Molecule-1 (VCAM-1), a protein present on inflamed endothelial tissues that attracts leukocytes to bind to binding sites (Gorelik et al., 2012).

An intraarterial microcatheter is introduced into the internal carotid artery, and contrast-enhanced MRI is used to optimize the area of cell distribution. The contrast agent-Ferehen makes the distribution area visible under MRI. When the injection speed is fast, the area of cell distribution is broad; when the injection speed is slow, the area of cell distribution is narrow. By adjusting the speed of infusion speed, the size of the area of cell distribution is matched to the size of the area of damaged tissue (Walczak, 2014; personal communication). After a few tries, the best injection condition is determined, along with optimal infusion speed that matches the size of cell distribution area indicated by the contrast to the size of the damaged area.

The engineered GRPs are then loaded into the syringe, connected to the microcatheter, and infused to achieve the best engraftment result. The engineered GRPs go through the blood vessel wall via opened tight junctions, and integrate with neural tissue, forming new myelin-producing oligodendrocytes.

Problem Statement

The importance of using glial progenitors for treatment of radiation brain injury and of improving the cell delivery procedure are under appreciated, and need more attention and financial support so that research can continue to progress, resulting in effective
clinical trials with ultimate benefit to patients. However, the complex scientific nature of the subject, makes it difficult to explain the experimental and clinical approaches to the lay audience in a comprehensive manner.

The internet search yields only one animation on this topic. There is a silent animation, of 1 minute 8 seconds duration, showing the results of Pioa et al. (2015). “Human Embryobic Stem Cell-Derived Oligodendrocyte Progenitors Remyelinate the Brain and Rescue Behavioral Deficits Following Radiation” (published in Cell Stem Cell in February 2015). The animation shows the growth of oligodendrocyte progenitors in vivo. However, the animation does not explain the mechanism of the cell delivery methodology nor cell modification, and refers only to local cell delivery rather than the intraarterial methods proposed by Dr. Walczak. Furthermore, the Pioa et al., (2015) animation used low resolution 3D models derived from cellular data, an approach that makes it hard for the lay audience to understand the oligodendrocytes and myelin are regenerated, and the neurological condition has been improved.

Thus, it appears that there is no animation that clearly and properly communicates, for the lay audience, research on intraarterial transplantation of GRPs to regenerate area of the brain that are damaged due to radiation therapy. This deficit needs to be addressed because of the great clinical potential of this new approach.

**Project Objectives**

For this project, an animation is being created to effectively communicate, to the lay audience, the promising approach for regeneration of the damaged CNS that is being developed by Dr. Walczak’s research lab. The animation should inform members of the public, and individuals in government and the private sector who may provide financial support. The animation will also be a key teaching tool to share with research colleagues.
The goals of the animation are to: (i) accurately show methodology for transplantation of engineered glial progenitors, and (ii) present the information at a level suitable for the general public and for scientists. The animation will focus on stem cell transplantation for restoring lost function of the central nervous system.

The specific objectives are to explain: (i) What glial restricted progenitors are, and how they can be engineered to enhance their therapeutic potential, (ii) methodology of using MRI for guidance to effectively and safely deliver GRPs to the targeted site, and (iii) mechanism of improved neurological function in rodents and perspectives for clinical translation.
MATERIALS AND METHODS

Creating The Script

To gather information detailing radiation-induced injury and treated with glial progenitors as cell transplantation treatment, research papers and review articles supplied by Dr. Walczak were reviewed. Texts on neural histology were also researched. A literature search of overall background knowledge related to the topic included the following keywords: brain tumor radiation treatment, side effects from brain tumor radiation treatment, neural forest, oligodendrocyte, VLA-4, and stem cell transplantation treatment for neurological disease. In addition, meeting with Dr. Walczak and researchers working in his lab also contributed to the development of a script for the animation.

Once the engineered glial progenitors and the transplantation procedure were understood, several versions of script drafts were developed and discussed with Dr. Walczak, my thesis advisor Mr. Tim Phelps, and my classmates. Their comments and recommendations were incorporated into the final script.

The primary intended audience for the animation are the general public and scientists, therefore clarity and information accuracy are critical. The content has to be clear and easy for the general public to understand, in order to raise their interest on the topic. The content has to be accurate, covering key points of the complex subject, and the procedure, for scientists.

The script is the backbone of the animation; it is broken down into three sections of information: introduction, pre-clinical study on rodent models, and clinical potential.
Materials and Methods

**Storyboards**

The storyboard is the framework for the animatic and animation. The initial storyboard (Appendix B) served as a basis for discussion, and was developed using the draft script and graphite sketches. The storyboard was discussed with Dr. Walczak to form a more concrete script. Once the finalized script (Appendix C) was developed and approved, more refined storyboards were completed.

The final images were drawn in graphite, digitized, and then edited and colorized using Adobe Photoshop CC 2014. The action of the elements in the frame, and the narration, were noted adjacent to each frame. Research on 3D animation (Appendix D) explored camera angles and compositions from demo reels from 3D biomedical animation companies. These reels were used as stylistic inspiration for the use of color, motion, and composition within the storyboards.

**Narration**

Narration, provided by Ms. Dacia Balch, was recorded professionally at the Johns Hopkins School of Medicine Departments of Medical Video. Two versions of the voiceover were recorded: version1 and version2. Both versions were delivered digitally in .wav files, and these two files were then edited in Adobe Audition CC 2014 to be composed into the final version. Unwanted portions of the narration were deleted, background noise was toned down, and stretch (rate of speech) and pitch were adjusted (Figure 1). The narration had a word count of 692, and the duration was 5 minutes 31 seconds. The word per minute rate was adjusted to the 120-150 words per minute suggested by Sinsel (2013) in her thesis: ““Visualizing the Sexual Stages of Plasmodium falciparum: Gametocytesgenesis and Targeted Interventions”.”
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Figure 1. Screen capture of Adobe Audition showing how to adjust ‘Stretch’ to increase or decrease the rate of speech, and adjust the ‘Pitch Shift’ to lower or raise the pitch of speech.

Cue markers, with keywords labeled on the marker’s name, were added as references for the development of the animatics and animation (Figure2).
Figure 2. Screen capture of Adobe Audition showing the addition of cue markers to add assigned keywords on the script to provide key frame reference for animatics and animation.

**Animatic**

To create the animatic (a set of animated storyboards), the software Adobe After Effects CC 2014 was used. The edited audio file and images from the storyboard were imported. Composition and motion of images were matched to markers on the audio file to achieve the effects delineated in the storyboards. Dr. Walczak and thesis adviser Mr. Tim Phelps reviewed the animatic, and offered suggestions for editing the animation. Changes were identified in the process when modifications are easy to make.
Creating assets

2D elements:

To communicate effectively with the intended audience, 2D elements were designed to be simple and didactic, and to include sufficient information to illustrate the major concepts identified in the storyboard. The 2D elements were drawn in graphite, scanned to the computer, and imported into Adobe Illustrator CC 2014. Clear contour and shapes were created using the “Pencil tool” and “Pen tool”, and then exported to Adobe Photoshop CC 2014, where areas of flat shadow were added (Figure 3). The animation had a HD ratio of 16:9, and a resolution of 1920 x 1080 pixels, therefore resolution of 2D images completed were no smaller than final image size of 1920 x 1080.

Figure 3. Screen capture of 2D images in Adobe Illustrator and Adobe Photoshop. Ventral view of rodent shows brain, heart, lungs and large vessels through which the GRPs are delivered. Image on left indicates a concise contour developed in Adobe Illustrator, right image after tone is added to clarify most salient information.
3D elements:  

--Rodent brain slice-from 2D image to 3D model

A reference image of the coronal section of a rat brain showing vessels (Taheri & Sood, 2006) was found by typing the search words “rat brain arteries” into the ClinicalKey search database, with filter by “images”. The reference image served as a basis, in Adobe Illustrator CC 2014, for creating a concise contour by using “Pencil tool” and “Pen tool” (Figure 4). This path was saved as an Illustrator 8 file was named “rat brain slice 3d”

Figure 4. (Left): Reference of a rat brain slice from Thera and Sood (2006) “Partial volume effect compensation for improved reliability of quantitative blood-brain barrier permeability” (Right): The vectorized shape of the reference image made in Illustrator, ventricles are represented by the space in white.

The “rat brain slice 3d” Illustrator 8 file was then brought into Cinema 4D by Clicking “Merge” and selecting the file, thus creating a new file. The imported AI path was converted to a “Spline Object” in Cinema 4D (Figure 5)

Figure 5. Screen capture. Illustrator 8 file “rat brain slice 3d” was merged into Cinema 4D as a spline object.
The depth of the 3D rat brain slice was created, making “Spline Object [rat brain slice 3d]” a child of an “Extrude Object” (Figure 6). The tissue depth was adjusted by changing numbers in “Movement” under “Object properties” in Extrude Object menu. The roundness of the edge was created with the “Caps and Rounding” by selecting “Fillet Cap” from dropdown menu on both Start and End, 3 steps and 2 cm radius were applied (Figure 7).

Figure 6. Screen capture of Cinema 4D. The depth was created by using an “Extrude object” generator, and placing the imported spline object as the child of the generator (highlighted in red circle).
The color and location of the blood vessels in the rat brain slice were added by creating a texture in “material”. A .jpg file was created using the same work flow described in the 2D elements section (Figure 8). The 2D image was projected on the 3D model by default as UWV Mapping. The mapping changed “UWV Mapping” from the projection dropdown menu under “Texture Tag” to “Flat” (Figure 9). The size of the
Materials and Methods

texture image projecting on the model was adjusted by changing the percentage on both Length U and Length V. The ideal placement of the texture image was achieved using the move tool while in “Texture Mode” (Figure 10).

The 3D models of the human spinal cord slice and the human brain slice used in the animation were also created using this method.

Figure 8. (Top) Rat brain texture and blood vessels. (Bottom) Screen shot of Cinema 4D material editor. The 2D texture .jpg file that was created in Illustrator and Photoshop was loaded for “Texture” under “Color” in material editor.
Figure 9. Screen capture of Cinema 4D. The orientation of the texture projection was adjusted under “Projection” in “Texture Tag”. The “UVW mapping changed to “Flat”. 

Figure 10. Screen capture of Cinema 4D. The size of the texture image was adjusted in Length U and Length V. The placement of the texture image was adjusted by dragging the image under “Texture mode”.
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--Neuron-3D modeling

Reference images of neurons were obtained from print and online sources. The model of the neuron was separated into three parts: (i) neuron body with several dendrites, (ii) long axon, and (iii) myelin sheaths and nucleus.

Creating neuron body with dendrites

An 18 segment icosahedron sphere was made editable in Cinema 4D. Then 16 polygons were selected using the live selection tool while holding the “Shift” key, to allow multiple selection, and the “3” key to allow the sphere to be rotated. These 16 polygons were selected as the spots where dendrites would protrude from the neuron cell body. The 16 polygon selection was saved as a polygon selection tag for later use, by clicking “Set Selection” from “Select” dropdown menu (Figure 11). The protrusion was created by making “MoExtrude” (Motion Graphics Extrude Deformer) a child of Sphere and dragging the orange triangle: polygon selection tag to “Polygon Selection” (Figure 12).

Ideal curvature and length of dendrites was achieved by creating a spline, and dragging the points of the free form deformer (FFD) cage. The arrangement of the spline, sphere object, FFD deformer, and MoExtrude Mograph is shown in Figure 14. The deformers were made permanent by applying to the computer generated object, by selecting all elements and clicking “Current State to Object” (Figure 15 A).
Figure 11. Polygon selection tag was created by selecting polygons (shown in yellow) and clicking “Set Selection”.

Figure 12. Extrusion was created by dragging Polygon Selection Tag into “Polygon Selection” in Object Properties of MoExtrude.
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Figure 13. Screen capture of Cinema 4D. In Transform, P and Z were changed to 50 cm; S.X, S.Y, and S.Z were changed to 0.3; R.H, R.P and R.B remain the same at 0 degree.

Figure 14. Screen capture of Cinema 4D. The pyriform shape of the cell body was adjusted by dragging the points shown in yellow to ideal placement. The panel at the right shows the arrangement of the elements including objects, Mograph, and deformer.
Creating the long axon

The long axon was created using the “Sweep” generator as follows. First a freeform “Spline” was created in Right View window, as well as a circular spine with a radius of 10 cm. “Sweep” was selected from generator menu, and then both the “Circle” and “Spline” were dragged under “Sweep” and children were made. The plane of the circle was changed to XY, to make the axon a long hollow cylindrical structure. Curvature of the spline determined the curvature of the axon, the radius of the circle determined the thickness of the axon. Both start cap and end cap were set to “none” to create the opening on end surfaces for later use. At this point, “Spline”, “Circle”, and “Sweep” were selected, and the deformer was made permanent by applying “Current State to Object”.

Connecting neuron cell body with long axon

The neuron cell body and the axon were selected as two separate objects, and then made into a single object by clicking “Connect Object + Delete” (Figure 15 B).

An opening was created by deleting a polygon from the bottom of the neuron cell body, and connecting this opening to the open end of the axon by using “Create Polygon” (Figure 16) to link coordinating points to form new polygons. By doing this, the neuron cell body and the long axon were jointed, and made into a 3D neuron cell model. The smoother curves and finer details on the final neuron model were achieved by adding “Subdivision Surface” generator to the neuron model.

Figure 15. Screen capture of Cinema 4D. A: “Current State to Object” was accessed from “Conversion” under Mesh. B: “Connect Objects + Delete” was accessed from “Conversion” under Mesh.
Creating myelin sheaths

The string of myelin sheathes that cover the long axon was created using “Cloner” found under MoGraph dropdown menu. A “Cylinder” was made into correct size by adjusting “Object Properties” and “Caps” setting as shown in Figure 17. This “Cylinder” was then dragged as a child of “Cloner”. To achieve the ideal placement and number of cylinders to represent realistic myelin sheathes, the “Object Properties” settings of the “Cloner” were changed (Figure 18). The Mode was set to “Object” and the spline was entered into the object field to create the curvature for axon. By doing this, the string of clones followed the same curvature as the axon to create the look of myelin sheathes surrounding the axon. The Count was set to 95 to achieve ideal spacing in between myelin sheathes. “Offset” was set to -8% to create the ideal start point of the sheaths.
From single neuron to neuron forest

A sphere with a radius of 20 cm was created and relocated inside the neuron cell body and named Nc for the nucleus of the neuron. Once the single neuron was made, a “Null Object” was created and named as “Neuron”. All elements created to this point were selected and dragged into ”Neuron” (Figure 19) null object for effective organization and easy access. A camera was set. The Neuron null was duplicated 8 times, and names where assigned as Neuron1, Neuron2, and so on, through to Neuron9 (Figure 20)

To create a neuron filled environment matching #6 in storyboard, the placement and the angle of neurons were adjusted by the move tool and the rotation tool.

Figure 17. Screen capture of Cinema 4D. The cylinder perimeter settings for myelin sheaths: radius was set as 24 cm, height was 250 cm, height segments as 3, rotation segments as 24, and +z for orientation. Caps was set for 1 segment, with fillet setting to 8 cm as the radius and 4 segments.
Figure 18. Screen capture of Cinema 4D. Setting underlined with red lines were changed to create the look of myelin sheaths surrounding the axon.

Figure 19. Screen capture of Cinema 4D. (Left) The single neuron cell with myelinated axon. (Right) Organization of the elements including the null object named Neuron.
Materials and Methods

Figure 20. (Above) Sketch from storyboard #6. (Below, left) Composition of the neuron forest was adjusted to match the drawing in storyboard #6. (Below, right) Organization of objects.

Mature oligodendrocyte-3D modeling

The protruding processes of the oligodendrocyte were created in the same manner as the dendrites were created. The oligodendrocyte was moved near the axon dense area, matching the composition of #7 in the storyboard. The end of the processes were modified by moving tool to create a more realistic look of myelin sheathes. A helix was drawn and extruded using an Extrude Generator. Both the helix and generator were
selected, and “Current State to Object” was selected to make the extrude permanent, and to make it an editable object. The thickness of the rotating myelin sheathes was created using “Cloth Surface” (Figure 21). This spiral myelin sheath was then duplicated.

A myelin sheath was relocated near one of the protruding processes. The oligodendrocyte cell body and the myelin sheath were both selected, and made into one object by clicking “Connect Objects + Delete”. The myelin sheath and the oligodendrocyte process were joined by creating an opening in each object, and then connected using “Create Polygon”. The “Bulge” deformer was used to create an organic look on the connection between the flap of the sheath and the cylindrical process (Figure 22). These steps were repeated seven times to make the seven processes connect to the spiral myelin sheathes.

Figure 21. Screen capture of Cinema 4D. The “Cloth Surface” was accessed from “Cloth” under the simulate dropdown menu.

Figure 22. The smooth curve of the connection between an oligodendrocyte process and a flap of the myelin sheath was achieved by using “Bulge” deformer shown in pale purple, in the curve edge box on the left, and circled in red on the right.
Materials and Methods

Materials of the neuron forest

Colors were applied to the models and the environment using the Material Editor. The Material Editor contains multiple channels and options to determine the appearance of the model (Figure 23). By selecting the appropriate combinations of settings, realistic models and environment can be created. The materials used in the neuron forest are shown in Figure 24.

Material [Glass-Murano] is one of the built-in materials provided with Cinema 4D, and was applied to the neuron bodies. The yellow appearance of the myelin sheaths and oligodendrocytes was made with the color modified Material [Glass-Murano]. The purple color appears on the nucleus of the oligodendrocyte as a Material [Mat] with modified color channel, and a purple Fresnel texture in the luminance channel.

Figure 23. Screen capture of Cinema 4D. A: Channels available in Material Editor. B: Options can be edited under color channel.
Materials and Methods

![Figure 24. Screen capture of Cinema 4D. Materials used in neuron forest.](image)

**Animating 3D**

The 3D animation was created in Cinema 4D, by moving the camera to capture the designated composition of the view. The timing to show the action of elements, and frame numbers, was indicated by frames of the animatic and marks on the narration audio file. The position of the camera was adjusted with the “move tool”, and the “rotation tool”. Two methods, Camera Key Frame and Camera Morph, were used to create and control movements of cameras in the neuron forest theme in the introduction.

**Camera Key Frame:**

One camera was created, and the key frame was set at the ideal time. Next, the “move tool” and “rotation tool” were used to move the camera to the location that showed the composition matching the animatic, to set the next key frame. At this point, two key frames and two locations were set for the camera. A curved dotted line was generated depicting the path of the moving camera. Each dot on the path represented the location of the camera at every frame in between the two manually set key frames. To change the curve of the dotted line, “Auto Tangents” and “Clamp” were unchecked. The two handles away from the orange dot that appeared after deselecting “Auto Tangents”
Materials and Methods

and “Clamp” gave the freedom to adjust the curve to give the desired camera movement (Figure 25). The Camera Key Frame method gives great control to the placement of the camera, and allows smooth movement of the camera. The online tutorial “Cinema 4D Camera Animation Tips, Tricks, and Tags for 3D Camera” recorded by Sean Frangella shows a straightforward demonstration (Frangella, 2014).

![Figure 25. Screen capture of Cinema 4D. (Left) Image shows handle that adjusts the camera move track. (Right) Red underline shows that “Auto Tangents” and “Clamp” were unchecked.](image)

**Camera Morph:**

Two cameras were created and adjusted to capture the designated composition and view angles from cameras matched the composition and view angles in the animatic. From the objects panel, both cameras were selected, and then a Camera Morph was set by clicking Camera Morph from the camera menu (Figure 26).

![Figure 26. Screen capture of Cinema 4D. Camera Morph in camera menu.](image)
Selected cameras were automatically assigned as camera 1 and camera 2 in Camera Morph. The view panel was set to the under Cameras option, and Morph Camera was selected for “Use Camera” so the view panel showed the synchronized view of a given percentage blend (see blend in orange color in Figure 27). A 0% blend showed the view captured by camera 1; a 100% blend showed the view captured by camera 2. Any value in between 0% and 100% showed a blend between two cameras; if the number was nearer to 0%, the view was closer to camera 1 view; if the number was closer to 100%, the view was closer to camera 2. When ideal view was found, a key frame was set by
control-clicking the circle in front of Blend. The circle then turned to red when the key frame was set successfully. Camera Morph was a very useful tool for setting complex camera movements.

The online tutorial, “Creating Amazing Camera Moves Using the Camera Morph Tool” (A Cinema 4D Tutorial) recorded by Iain Chudleigh has an easy-to-follow demonstration (Chudleigh, 2014).

Cinema 4D render

Render settings were set using the “Physical Renderer” for all 3D scenes and assets. Dimensions were set to a high definition ratio of 1920 pixels for width, and 1980 pixels for height. Resolution was 72 DPI, frame rate was set to 30 frames per second. The same frame rate setting was later set in Adobe After Effects file. The Alpha channel was selected so that the background would be transparent, and images would be ready for later compositing in After Effects. The format was rendered as 16 bit PNG files. To allow for more detailed and realistic renders, Ambient Occlusion was applied from Effects.

Global illumination was only applied to demyelination and the damaged dark part of the cell neuron, due to a significantly longer render time. To keep the animation production process moving smoothly within a limited time, excessive render time should be avoided. These two parts were rendered as still images from selected frames. The animation of these two parts was composited and adjusted in After Effects. After scenes and render settings were completed, they were set to render using Cinema 4D NET Render Client for team render.
Materials and Methods

Compositing

Rendered image files of 3D scenes were imported into Adobe After Effects as a PNG sequence. Scenes were dragged to play at designated time points, as outlined in the animatic. Additional adjustment of opacity was applied for “fade on” and “fade off” to achieve smooth scene transition and blending in with 2D animation scenes.

Animating 2D

Photoshop files were imported as compositions and preserved layers in After Effects. Layers were placed to show the composition as it was designed in the animatic. Position, scale, rotation, and opacity were adjusted accordingly. In addition, mask was applied in some layers. Labels, and leader lines were also added in After Effects.

There were many layers used in the After Effects, and at one point the layers were hard to find and access. To address this, Pre-Compose was used, which is a powerful way to organize and handle a group of layers. Layers that appeared at the same time were selected, and right clicked to select “Pre-Compose” from the menu. The group then appeared as one “Comp” that the “Transform” option could apply to the whole grouped layer, yet, the individual layers still were accessible by double clicking the “Comp” as a composition. An online tutorial, “After Effects Tutorial-How to Pre-Compose-Level: Beginner” offered a detailed and easy to follow demonstration (SimpleMoGraph, 2013).

After Effect Render

The entire animation was divided into 3 compositions: Comp1 Intro, Comp2 preclinical, and Comp3 Clinical potential. An additional composition, Group Comp, was made for composition nesting. After Comp1 Intro, and Comp2 preclinical were completed, Comp1 and Comp2 were dragged to the Group Comp. All audio was unchecked except Group Comp, to make sure the animation was not rendered with more
than one copy of narration. The Group Comp was then added to Render Queue. The settings were: best for quality, full for resolution, size 1920 x 1080 for Render Settings; QuickTime for format, RGB for channels, millions of colors for depth, and audio output auto for audio in Output Module. After this, the After Effects Project file was rendered as .mov file, and was then dragged to Adobe Media Encoder CC 2014. Start Queue was clicked to turn this 13.5 GB .mov file into a 311.5 MB, much smaller, and accessible .mp4 file.
RESULTS

Goals of the project

The primary goal of this project is to produce an animation to educate a lay audience and scientists about the new concept of applying regenerative medicine solutions in oncology. Specifically, the aim is to provide an overview of the intraarterial transplantation of glial restricted progenitors (GRPs), and their ability to regenerate brain tissue following its damage during radiation therapy for brain tumors.

The animation integrates both 2D and 3D elements, and shows the principles of this promising central nervous system (CNS) regeneration approach, to the audience in a comprehensive manner. The intent is to increase interest in this interdisciplinary approach, and hopefully thereby encourage funding for future research, and ultimately lead to more effective and safer therapy of brain tumors.

The goals of the animation are to:

(i) accurately show methodology related to engineering and transplantation of GRPs, and

(ii) present the information at a level suitable for both the general public and scientists.

Preparation for the animation

For developing the animation, the following materials were prepared: (i) a finalized script for narration (Appendix C), (ii) a finalized recorded narration, (iii) an initial storyboard (Appendix B), (iv) a finalized storyboard (Appendix E), and (v) an animatic. More details are given in the Materials and Methods section.
Animation

The first portion of the animation: Introduction, begins with statistical data showing the number of patients suffering from the damage caused by brain tumor radiation therapy (Figures 28, 29). The next section of the introduction shows the basic anatomy of the CNS (Figure 30, 31), the physiological role of myelin producing oligodendrocytes (Figure 32), and their functions (Figure 33). Later, the principles of demyelination are introduced with reference to how it affects patients who have undergone brain tumor radiation therapy (Figures 35-37). Concepts are explained subsequently, so that the audience who is not familiar with the subject, can learn the most relevant and important facts while watching the animation, and understand the mechanism in which transplanted GRPs can repair radiation mediated demyelination.

The second portion: Pre-clinical study on rodent models (Figure 38), initially discusses the key problem of effective delivery of cells to the area of brain damage (Figure 39). A traditional cell delivery approach is inefficient in the case of large brain lesions, because the cell distribution from a needle injection is not broad enough to cover the damaged site (Figure 40). An improved approach, intraarterial cell delivery, offers broader cell distribution than the needle approach.

The animation then introduces the concept of engineering GRPs, and using the intraarterial approach, which is a novel and auspicious solution provided by Dr. Piotr Walczak at the Institute for Cell Engineering (ICE) of Johns Hopkins Medicine (Figure 43).

However, there are drawbacks to the intraarterial approach, related to either insufficient or excessive accumulation of GRPs. Cells that do not adhere at the targeted site are lost to filtering organs such as the lungs, and excessive cell accumulation may result in stroke (Figures 41, 42). The solution to these issues is two-fold: (i) control cell binding to the brain blood vessels with overexpression of adhesion molecule VLA-4, and
(ii) label the cells with iron oxide nanoparticles, to make the cells visible under MRI, and then use MRI for monitoring the cell transplantation procedure. This approach provides tools for more precise and safer delivery of cells to the targeted site (Figures 44-47).

After the optimal injection parameters are determined, the engineered GRPs are injected (Figure 48-50). Ultimately the goal is for engineered GRPs to remyelinate the demyelinated axons, and regenerate brain tissue following radiation therapy of brain tumor (Figure 51-55).

![Figure 28. Animation screen capture: Introduction; Statistical data.](image)

![Figure 29. Animation screen capture: Introduction; Radiation induced brain injury](image)
Figure 30. Animation screen capture: Introduction; Central nervous system, brain, and spinal cord.

Figure 31. Animation screenshot: Introduction; Neuron cell.
Figure 32. Animation screen capture: Introduction; Oligodendrocyte

Figure 33. Animation screen capture: Introduction; Normal nerve function: feel, think, move.
Figure 34. Animation screen capture: Introduction; Glial restricted progenitor.

Figure 35. Animation screen capture: Introduction; Demyelination.
Figure 36. Animation screen capture: Introduction; Continuous demyelination.

Figure 37. Animation screen capture: Introduction; Neural damage leads to problems with feeling, thinking, and moving.
Figure 38. Animation screen capture: Pre-clinical study on rodent models; Title page.

Figure 39. Animation screen capture: Pre-clinical study on rodent models; Rodent use for therapy study.
Results

Figure 40. Animation screen capture: Pre-clinical study on rodent models; Limitation of traditional cell delivery approach, its small distribution area for the cells.

Figure 41. Animation screen capture: Pre-clinical study on rodent models; Possible problem from intraarterial approach, cells shown in green do not adhere at the targeted site.
Results

Figure 42. Animation screen capture: Pre-clinical study on rodent models; possible problem from intraarterial cell delivery approach, stroke and adhering in lungs are possible issues.

Figure 43. Animation screen capture: Pre-clinical study on rodent models; photograph still for Institute of Cell Engineering (ICE).
Results

Figure 44. Animation screen capture: Pre-clinical study on rodent models; Iron oxide particles enter the GRP. The GRP is spindle shape in vitro.

Figure 45. Animation screen capture: Pre-clinical study on rodent models; Iron oxide labeled GRP and MRI machine.
Results

Figure 46. Animation screen capture: Pre-clinical study on rodent models; Adhesion molecule VLA-4 enters the GRP.

Figure 47. Animation screen capture: Pre-clinical study on rodent models; Engineered GRPs in the brain blood vessel are stopped by attraction between VLA-4 and VCAM-1, and adhere to the inflamed endothelial vessel wall at the targeted site. GRPs are ovoid shape in vivo.
Figure 48. Animation screen capture: Pre-clinical study on rodent models; Contrast agent injected slowly results in a narrow distribution (shown in blue). Volume was the same, only speed changed.

Figure 49. Animation screen capture: Pre-clinical study on rodent models; Contrast injected rapidly results in a broad distribution (shown in blue). Volume was the same, only speed changed.
Results

Figure 50. Animation screen capture: Pre-clinical study on rodent model; Injecting engineered GRPs.

Figure 51. Animation screen capture: Pre-clinical study on rodent models; engineered GRP passes out through the blood vessel wall.
Figure 52. Animation screen capture: Pre-clinical study on rodent models; Engineered GRP in the brain parenchyma.

Figure 53. Animation screen capture: Pre-clinical study on rodent models; Engineered GRP grows into an oligodendrocyte with foot-like processes forming new myelin sheaths that integrates with neural tissue.
Figure 54. Animation screen capture: Pre-clinical study on rodent models:
A mature myelin-producing oligodendrocyte and remyelinated axons.

Asset Referral Page

Access to the animation resulting from this thesis is available at
http://www.hopkinsmedicine.org/profiles/results/directory/profile/5840468/piotr-walczak,
or by contacting the author at student’s email address: wuihsun@gmail.com

The author may also be reached through the Department of Art as Applied to Medicine via the website www.hopkinsmedicine.org.medart
DISCUSSION

**Media format**

Multimedia enhances the quality of learning compared to tradition learning settings. (Leow, 2014; SEG Research, 2008). Thus, an animation is the best option as media format for this project, to allow the selected audience to learn the complex information on this subject in a short time.

The animation, including 2D images and 3D elements, visualizes concepts that may be difficult for a lay audience to understand. Studies show that animations are more effective if accompanied by narration, making use of both the auditory and visual channels (SEG Research, 2008). Therefore, this animation, and carefully designed and integrated narration, provides an efficient overview of intraarterial transplantation of glial restricted progenitors (GRPs), and their ability to regenerate brain damage following radiation therapy for brain tumors.

**Storyboard**

A well-known idiom states that, “A picture is worth a thousand words”, and this project, as an animation, conveys complex ideas, and extensive knowledge from current research, in 4 minutes and 40 seconds. There are four ways to effectively organize the narration and images in an animation: defining, comparing, sequencing, and finding causes and effects. Selected examples of these are now discussed.

Defining:

-Storyboard #4–#8: The central nervous system, brain, spinal cord, grey matter, white matter, structure of a neuron including cell body and axon, oligodendrocytes, and myelin sheaths, are each defined and labeled at key moments of the narration.

-Storyboard #12 or Figure 35: Demyelination is defined.
Comparing:

-Storyboard #10 vs. #15 (or Figure 33 vs. Figure 37): The image composition of these two frames are similar, the icons compare the affects from healthy brain with myelinated axons and damaged brain with demyelinated axons.

-Storyboard #22: Cell distribution from the traditional needle approach and the intraarterial approach are compared.

Sequencing:

-Figure 34-35: Demylination is described and demonstrated in sequence.

-Figure 51-55: The transplanted engineered GRPs migrate out from a blood vessel, and grow into mature myelin-producing oligodendrocytes and repair demyelinated axons.

Finding causes and effects:

-Figure 36: Radiation therapy for brain tumor patients may cause demyelination, and that process is illustrated.

**Animatic**

The animatic is indispensable for the production of this animation. An animatic is the most efficient way to adopt still images from storyboard, and combine them with the narration audio file, to illustrate the concepts of cell engineering and transplantation. Devoting time to creating a more complete animatic can save time in producing the final animation. The animatic also determines the correct timing for actions of assigned elements, and identifying the numbers of key frames when specific events happen.

For instance, when the narration says, “The myelin protects the axon, and insulates the electrical impulses to allow neurons to transmit impulses quickly and efficiently, so we can feel, think, and move the way we normally do”, the view of the video zooms out, showing the “neuron forest” with pulsating light bulbs traveling along axons. After imported the still images from the storyboard #9 and #10, and matching the
narration audio file in Adobe After Effects, the file showed images appeared from frames 1510 to frame 2093 give the best result. Therefore, the 3D scene of “neuron forest” with fast moving light bulbs is indicated in the following manner (2093-1510=583) so this scene is 583 frames long.

In this project, one frame in 3D scene takes an average of 3 minutes to render. With the frame set at a rate of 30 frames per second, a scene of 583 frames is about 19.5 seconds long in the final animation. This 19.5 seconds 3D scene may take almost 30 hours to render on a computer. While the team render for this project took 12 hours, which is much shorter than 30 hours, it is still considered a time consuming process. Therefore, planning ahead, especially knowing the exact number of frames that is essential for the final animation, is one of the ways to use time more economically. The key frame reference provided by the animatic is a time-saving device.

**Animation**

Using didactic color to bind 2D and 3D elements:

One of the challenges of making an animation combining both 2D and 3D elements is that there are differences in style. To give the animation a coherent appearance, both conventional colors and didactic colors were used for a realistic look, building a visual information linkage between 2D images and 3D elements.

Color is a potent tool in visual communication. Neuronal bodies (Figure 31) were made in light blue which echoes with the human contour (Figure 30). Myelin sheaths, and oligodendrocytes were made in yellow (Figure 32), which echoes the color choice of the brain and spinal cord in the human figure (Figure 30).
Discussion

In the introduction, a light blue color serves as landmark and map where the story takes place, and is common enough for lay audience to recognize. The color yellow serves as a highlighted point that shows the structures as a key character that demanding audience attention. As another example, green is used for non-engineered GRPs. Green appears in Figure 34 for a 3D rendered GRPs, and in Figure 40-42 as showing the distribution of the non-engineered GRP cells.

Using 3D rendered stills for static scene:

When the view is static, using a rendered still image from a 3D scene, and imported to Adobe After Effects, can achieve the desired effect that is assigned in the storyboard for the final animation. The demyelination and remyelination scenes were produced in this way. When this approach is used, the 3D render time is shorter than rendering the whole scene in 3D render. In the demyelination scene, 333 frames were requested. Four layers of still images from this scene were rendered, taking 15 minutes. If 333 frames were all rendered in Cinema 4D, it may take 333 minutes when one frame takes one minute to render; 999 minutes when one frame takes three minutes to render.

The average time spent on rendering one frame in this project was three minutes. By taking the rendering 3D scene still images approach, 16.4 hours of render time was saved for the demyelination scene.

**Potential Improvement**

Because of the time constrains, a portion of the animation directly referring to Clinical Potential was eliminated. This portion may be added subsequently to further improve the clinical relevance and impact of this work.
Transplantation of glial restricted progenitors (GRPs) as a strategy to regenerate brain damage following radiation therapy for brain tumors is critical ongoing research. The approach presented in this animation is the most promising current approach. However, as our knowledge of cell biology, cell trafficking, and cell engineering expands, some components of the study or experiments will likely change in the future.

Future alteration will be needed to keep this animation current with the latest research discoveries. For instance, currently the lab uses VLA-4 to allow GRPs to adhere on the damage site; this adhesion molecule may be replaced or supplemented with other adhesion molecules when justified (Walczak, 2015; personal communication).
APPENDIX A

List of software

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adobe Photoshop CC 2014</td>
<td>Adobe Systems Inc.</td>
</tr>
<tr>
<td>Cinema 4D R15</td>
<td>MAXON Computer</td>
</tr>
</tbody>
</table>
The grey matter contains most of the neuron cell bodies, and the white matter contains myelinated axons.

In the CNS, nerve fibers are wrapped by plasma membranes of the oligodendrocyte forming myelin sheaths.

Audio

The myelin promotes the axon and insulates the neural impulses to allow neurons to transmit impulses quickly and efficiently.

Oligodendrocytes are derived from immature cells called glial restricted progenitors.

Video

Zoom out to view whole neuron with oligodendrocyte.

Light up segment running down when audio says feel, feel icon appears; when audio says think, think icon appears; when audio says move, move icon appears.

 Insets: zoom in to one neuron, and show detail structures in myelin sheaths.
When a myelin sheath is damaged, this is called demyelination.

Demyelination has been observed following a radiation-induced brain injury.

Once the myelin is damaged, it may lead to deterioration of the nerve and the neuron itself.

Show normal myelin sheaths on axons.

Show the case with damaged myelin sheaths when audio says "damaged..."

Show the light connect pass, and disappear at the damaged myelin sheaths.

Draw a cross to indicate the learning problem.

Researchers have been using transplants of neural progenitors to regenerate myelin in rodent models.

Show dead neuron, darker background and fade to black. Fade to black, transition to 20.

Blue neural progenitor appears first, then audio says "in rodent models".
Audio: Highly similar genes, biology and anatomy of the CNS in rodents and humans make rodent brains a great study tool for developing novel therapies for brain damage.

However, there are several challenges which have made glut projection template therapy infeasible in the past.

Our problem was different cell density throughout. Typically, cells are injected with needles and must utilize auxiliary neural distribution areas.

Video: Insert slide to the right with CNS lights on (blue), and fade out human head with human CNS lights on (blue) on the left where audio says "Humans".

When audio says challenges, challenge appears when audio says "impossible", the screen turns less saturated.

Images show in human or rodents?

Ask for reference image.

Audio: The arm arterial approach allows larger cell distribution.

However, it may cause other problems.

The first problem was that going straight at the wrong places in the body, a second problem was that our abundance proportion being denatured for example the lungs.

Video: Show artery network, microvessels; spread larger blue area.

Show blue move from head area to heart and to lungs by venous network.

Show high density of blue stop as an artery branch.
### Audio
- Could accumulate at a site in the vessel, potentially leading to micrometabolism.
- To address these issues, the cells need to be modified and monitored.

### Video
- Blue block the artery, tissue
- Micrometabolism image slide to the left, travel to lung image appears on the right.
- Transition from 34 to 36.
- Affiliation logo fade on, maybe a blurry lab photo as background.

### Audio
- Glial restricted progenitors are modified with nanoparticles called SPIO.
- MRI quickly zoom in and fade off.
- Show blue cells (or arrows) moving in rodent brain.
- Yellow VLA-4, and make blue GRP green.
Audio:

extravasate, and grow into oligodendrocytes forming new myelin.

Video:

This will regenerate the damaged brain tissue and improve neurological condition of brain tumor patients following radiation.

In children, improve their life quality.

Audio:

credit page

Video:

credit page
APPENDIX C

Final Script

Title of the animation:

Intraarterial Transplantation of Glial Progenitors to Regenerate Brain Damage Following Radiation Therapy of Brain Tumor

Introduction

In the U.S. each year, approximately 100,000 patients develop radiation-induced brain injury from brain tumor radiation therapy.

The central nervous system, or CNS, comprises the brain and spinal cord. The CNS is made up of grey matter and white matter: the grey matter contains most of the neuron cell bodies and the white matter contains myelinated axons. In the CNS, nerve fibers are wrapped by a varying number of concentric layers, with the plasma membrane of the oligodendrocytes forming myelin sheaths. The myelin protects the axon and insulates the electrical impulses to allow neurons to transmit impulses quickly and efficiently so we can feel, think, and move the way we normally do. Oligodendrocytes are derived from immature cells called glial-restricted progenitors.

When a myelin sheath is damaged, this is called demyelination. Demyelination has been observed following radiation-induced brain injury. Once the myelin is damaged, it may lead to deterioration of the axon and the neuron itself: these changes may lead to problems with feeling, thinking, and moving. If demyelination persists, it could lead to irreversible damage to neurons and loss of neurological function. Transplantation of glial-restricted progenitors has the potential to prevent this.
Pre-Clinical Study on Rodent Models

Researchers have been using animals to study the functions of oligodendrocytes. The CNS of rodents and humans is highly similar in biology and anatomy, making rodent brains a great study tool with which to develop new therapies for brain damage, such as radiation-induced brain injury.

However, therapy based on cell transplantation is challenging. The key problem is inefficient cell delivery methods. Typically, cells are injected via needles and that results in a very small distribution area. The intra-arterial approach allows broader cell distribution. But, this may cause other problems.

The first problem is that progenitors might not adhere at the targeted site. Instead, they would pass through and become trapped in the wrong places in the body, such as the lungs. A second problem is that an excessive adhesion and an overabundance of progenitors being delivered could cause them to accumulate at a site in blood vessels, which could block blood flow and lead to microembolism and stroke. To address these issues, the cells need to be modified and the injection procedure needs to be monitored with imaging.

In Dr. Piotr Walczak’s lab at the Johns Hopkins University Institute for Cell Engineering, glial-restricted progenitors are labeled with iron oxide nanoparticles, making cells visible under MRI to keep track of where the labeled cells are and what they do. In addition, an adhesion molecule, VLA-4, is used to make these progenitors adherent to areas of the damaged brain. An intra-arterial microcatheter is introduced into the internal carotid artery and contrast-enhanced MRI is used to optimize the area of cell distribution. The contrast makes the distribution area visible under MRI. The size of the cell distribution area is matched to the size of the damaged area by adjusting the infusion speed. We have determined the best injection condition along with optical infusion speed. The engineered glial-restricted progenitors are then loaded into the syringe,
connected to the microcatheter, and infused to achieve the best engraftment result. The engineered glial-restricted progenitors then go through the blood vessel wall via opened tight junctions and integrate with neural tissue, forming new myelin-producing oligodendrocytes.

Clinical potential

When using radiation therapy to destroy a targeted brain tumor, the brain tissue surrounding the tumor can also be damaged by radiation. The transplantation of engineered glial progenitors is an attractive solution for repairing radiation-induced brain injury. The microcatheter is able to target a brain area via a specific artery network that supplies the area with the radiation-induced brain injury. With MRI guidance, the engineered glial progenitors can be injected to the ideal site. These progenitors will adhere, go through the blood vessel wall to the damaged site, and grow into oligodendrocytes, forming new myelin. This may regenerate the damaged brain tissue and improve the neurological condition and quality of life for brain tumor patients following radiation.
APPENDIX D

Video style reference

1. The Schwann Cell and Action Potential
   JCCCvideo
   https://www.youtube.com/watch?v=DJe3_3XsBOg
   -reference for signal transmission in healthy neuron and demyelinated neurons

2. Multiple Sclerosis-Myelin Repair
   Myelin Repair Foundation
   https://www.youtube.com/watch?v=UzDPWrv8D2g&index=18&list=PLBC389D37F5
   CAF8A2
   -reference for demyelination, myeline regeneration

3. XVIVO
   http://www.xvivo.net/

4. Random 42
   http://www.random42.com/

5. Fusion Medical animation
   https://www.youtube.com/watch?v=9iP3MaiaMsg

6. Vessel studio
   http://www.vesselstudios.com/

7. Artery studio

8. useful brain cut website
APPENDIX E

1.
Intraarterial Transplantation of Glial Progenitors to Regenerate Brain Damage following Radiation Therapy of Brain Tumor

2.
Each year
100,000 patients

3.
Radiation-induced brain injury from brain tumor radiation therapy.
When audio says "brain", brain and label light up; when audio says "spinal cord", spinal cord and label light up.

The central nervous system, or CNS, comprises the brain and spinal cord.

When audio says grey matter, grey matter showes grey color; when audio says white matter, white matter shines white.

The CNS is made up of gray matter and white matter.

When audio says cell bodies, cell bodies light up; when audio says myelinated axons, axons light up.

The grey matter contains most of the neuron cell bodies and the white matter contains myelinated axons.
Video: Zoom in to one neuron and show detail structures including myelin sheaths.

Audio: In the CNS, nerve fibers are wrapped by a varying number of concentric layers.

Video: Camera move and zoom in to center the oligodendrocytes

Audio: With the plasma membrane of the oligodendrocytes forming myelin sheaths.

Video: Zoom out, light segment running down the axons in between myelin sheath, like jumping.

Audio: The myelin protects the axon and insulates the electrical impulses to allow neurons to transmit impulses quickly and efficiently.
When audio says feel, feel icon appears; think, think icon appears; move, move icon appears.

So we can feel, think, and move the way we normally do.

Fade on glial restricted progenitor and its label when audio says it.

Oligodendrocytes are derived from immature cells called glial-restricted progenitors.

Show the axon with damaged myelin sheaths (deterioration and then disappear) when audio says "damaged".

When a myelin sheath is damaged this is called demyelination.
Video: The head with radiation ray fade on when audio says "following radiation-induced brain injury" and then fades off.

Audio: Demyelination has been observed following radiation-induced brain injury.

Video: Shows light passes axon very slowly and even stops then disappears.

Audio: Once the myelin is damaged, it may lead to deterioration of the axon and the neuron itself.

Video: Feel: feel problem icon appears; think: think problem icon appears; move: move problem icon appears.

Audio: These changes may lead to problems with feeling, thinking, and moving. If demyelination persists,
Video: Show dead neuron, darker background and fade to dark

Audio: it could lead to irreversible damage to neurons and loss of neurological function. Transplantation of glial-restricted progenitors has the potential to prevent this.

Video: “Rodent Model” appears

Audio: Pre-Clinical Study on Rodent Models.

Video: Rat appears

Audio: Researchers have been using animals to study the functions of oligodendrocytes.
Rat and human with their brains fade on when audio says “rodents” and “human”.

The CNS of rodents and humans is highly similar in biology and anatomy, making rodent brains a great study tool with which to develop new therapies for brain damage, such as radiation-induced brain injury.

Challenge

Blue as needle injection distribution area.

The key problem is inefficient cell delivery methods.
Video: "Traditional approach" moves to right and fade on "Intra-arterial approach".

Audio: Typically, cells are injected via needles and that results in a very small distribution area. The intra-arterial approach allows broader cell distribution.

Audio: But, this may cause other problems.

Video: Intra-arterial approach zooms in and moves to the center of the frame.

Audio: Show blue cell injection distribution moves from brain area to heart and to lungs by venous network.

Audio: The first problem is that progenitors might not adhere at the targeted site. Instead, they would pass through and become trapped in the wrong places in the body, such as the lungs.
Blue cells block the vessel, tissue died (turn dark color)

A second problem is that an excessive adhesion and an overabundance of progenitors being delivered could cause them to accumulate at a site in blood vessels, which could block blood flow and lead to microembolism and stroke.

Microembolism image slide to left, travel to lung image appears on right.

To address these issues, the cells need to be modified and the injection procedure needs to be monitored with imaging.

Affiliation logo fade on, lab photo as background.

In Dr. Piotr Walczak’s lab at the Johns Hopkins University Institute for Cell Engineering.
Video: SPIO moves to GRP, GRP becomes red and clear (easy to see).

Audio: glial-restricted progenitors are labeled with iron oxide nanoparticles

Video: MRI machine (MRI 3T) zoom in and fade off.

Audio: making cells visible under MRI to keep track

Video: Show MRI with and without SPIO

Audio: of where the labeled cells are and what they do.
DNA shape VLA-4 enter GRP, yellow dots appears on GRP.

In addition, an adhesion molecule, VLA-4.

Engineered GRP travels in the vessel.

is used to make these progenitors

Engineered GRP stop, blue in lungs rat image fades on for contrast.

adherent to areas of the damaged brain.
Video: Syringe push slow, blue area smaller than red damage area.

Audio: An intra-arterial microcatheter is introduced into the internal carotid artery and contrast-enhanced MRI is used to optimize the area of cell distribution. The contrast makes the distribution area visible under MRI.

Video: Syringe push fast, blue area larger than red damaged area.

Audio: The size of the cell distribution area is matched to the size of the damaged area by adjusting the infusion speed.

Video: Blue area matches with damaged area.

Audio: We have determined the best injection condition.
**Video:** Red engineered GRP is injected to the rat brain slice.

The engineered glial-restricted progenitors are then loaded into the syringe, connected to the microcatheter, and infused to achieve the best engraftment result.

**Audio:** The engineered glial-restricted progenitors then go through the blood vessel wall via opened tight junctions.

**Video:** Red GRP exits vessel wall.

**Audio:** GRP grows into oligodendrocyte.

**Audio:** and integrate with neural tissue, forming new myelin-producing oligodendrocytes.
Clinical Potential

When using radiation therapy to destroy a targeted brain tumor, the brain tissue surrounding the tumor can also be damaged by radiation.

The transplantation of engineered glial progenitors is an attractive solution for repairing radiation-induced brain injury.
Show microcatheter goes in to the brain injury area via artery network, injecting red engineered GRP.

The microcatheter is able to target a brain area via a specific artery network that supplies the area with the radiation-induced brain injury. With MRI guidance, the engineered glial progenitors can be injected to the ideal site.

Red GRP adhere on the damaged site, exit the vessel wall.

These progenitors will adhere, go through the blood vessel wall to the damaged site.

GRPs grow into oligodendrocytes.

and grow into oligodendrocytes, forming new myelin.

This may regenerate the damaged brain tissue and improve the neurological condition and quality of life for brain tumor patients following radiation.
REFERENCES


VITA

I-Hsun Wu was born on July 19, 1987 in Yunlin County, Taiwan. She was educated from preschool to college in Taiwan, and was drawn to art and science at a young age. I-Hsun attended Taipei Municipal University of Education, and majored in Visual Art to explore the field of fine art. To advance her learning experience in scientific and medical illustration education, I-Hsun subsequently attended the Virginia Commonwealth University (VCU) in Richmond, Virginia, USA to pursue a Bachelor of Fine Arts in Communication Arts, with a Scientific and Preparatory Medical Concentration; she graduated in May for 2012 cum laude.

During her five years of study in VCU, I-Hsun’s artworks were honored by the Department of Communication Arts and displayed in the Anderson Gallery in a juried student exhibition for three consecutive years. I-Hsun received scholarships for two years at VCU, and Outstanding Scientific and Preparatory Medical Illustration Award in 2011, and Outstanding Communication Arts Medical Illustration Senior Award in 2012.

In August 2013, I-Hsun matriculated in the Department of Art as Applied to Medicine at the Johns Hopkins University School of Medicine. She is currently a candidate for a Master of Arts degree in Biological and Medical Illustration, to be awarded on May 21, 2015.

I-Hsun received an Award of Merit in the Student Didactic/ Instructional-Anatomical/Pathological category from the Association of Medical Illustrators in 2014.