Development of a Medical Animation on Acetaminophen Metabolism and Hepatotoxicity

Project Research

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I. Abstract

This project planned and created an animation on acetaminophen (APAP) metabolization with the intent of demonstrating its relationship to chemical-driven liver damage (hepatotoxicity). Although an APAP overdose has the potential to cause liver damage, it is a common ingredient in many prescription and over-the-counter medications. The goal of this project was to visually inspire interest in APAP metabolism while warning the viewer of the potential consequences to the liver if APAP is misused. Healthcare professionals could benefit from being more aware of how the medication works. The creation of an animation was the main focus of the project and no formal user testing of the animation was done. Project success was measured by completion of an animation that could be used to instill curiosity in the viewer while serving as a visual reinforcement of the recommended APAP dosage amounts. Information conveyed to the viewer through this animation may help prevent serious APAP overdose.

II. Overview

Acetaminophen (APAP) is an active ingredient of many widely used over-the-counter and prescription medications. Most healthcare providers consider APAP safe. The medicinal functions of APAP are to relieve pain and reduce fever (Tylenol®, 1999). Both of these functions are complex and are not entirely understood. Even what happens when APAP is metabolized remains uncertain. Since very serous side effects can occur if the recommended dose is exceeded, awareness of how APAP is metabolized should be brought to the attention of medical professionals (Larson et al., 2005). In 2009 a panel of the FDA recommended a reduction in how much APAP should be taken and provided usage warnings intended to protect the public.

If too much APAP is consumed, a build up of potentially hazardous metabolite byproducts could lead to liver damage (Gunawan & Kaplowitz, 2007). There is a fine balance between the medicinal amount of APAP and an unintentional overdose situation. Bringing attention of the viewer to the science involved in APAP metabolism may lead to an understanding of how an overdose could potentially damage the liver.

Various types of medical animations have been created such as anatomical, cellular and molecular. Molecular animations often show a mechanism of disease (MOD) or a mechanism of action (MOA). A MOD demonstrates a pathophysiology while an MOA could show certain treatments to the problem. MOA animations can be a powerful way to communicate complicated subjects to those in the pharmaceutical industry (Kermani, 2009). The combination MOD/MOA type animation created for this project was intended as an educational piece for an audience of health care professionals.

This project did not focus on the functional properties of APAP but instead concentrated on the metabolization and subsequent consequences to the body if too much is taken. The animation was created to dynamically introduce the APAP metabolic pathways, and visualize how overuse may lead to APAP-related liver failure. An animation on this topic could be used to educate and alert pharmacists, pharmacy technicians and other healthcare professionals on the potential dangers of APAP overuse. Understanding the science of APAP metabolism and overdose may help protect those who can benefit from its functional properties.

III. Literature Review

This literature review provides a brief overview of acetaminophen (APAP) metabolism and hepatotoxicity. The Tylenol® Professional Product Information (1999) and other research findings were examined and used for content development of the animation. How and why to educate healthcare professionals on overdose prevention depends on the needs of this audience. For the viewer to understand the problems associated with APAP, planning was done on what areas of information should be presented to them.

The approved usages of APAP are antipyretic (fever reduction) and analgesic (pain relief) (Tylenol®, 1999). The analgesic mechanism of action (MOA) of APAP is unlike anti-inflammatory drugs such as ibuprofen and aspirin used for similar conditions. Anti-inflammatories work to prevent the formation of prostaglandins that lead to inflammation (Graham & Scott, 2005). The MOA of APAP is unique. The main analgesic function of APAP is not anti-inflammatory but involves a decrease in pain signal transmission (Aronoff, Oates and Boutaud, 2006). APAP is a weak inhibitor of prostaglandin synthesis and that makes it different (Botting, 2000). The MOA for APAP analgesia is described in the Tylenol® PPI (1999) as an inhibition of the nitric oxide and neurotransmitters receptor pathways. In nerve cells, interruption of these pathways can elevate the pain threshold. APAP must first be metabolized in order for this interruption to occur.

Metabolites of APAP are produced during metabolization. Although some of these metabolites are part of an effective way to relieve pain, others have effects that can be non-beneficial (Graham & Scott, 2005). N-acetyl-p-benzoquinone imine (NAPQI) is one reactive metabolite produced. NAPQI is dangerous to cells and needs to be broken down further in to inert metabolites that can be disposed of by the liver (Tan, New, & Chan, 2008). This liver disposal system is in place as a way for the body to regulate not only what it consumes but also how it removes waste products. NAPQI can cause problems since the reactive metabolite can trigger a series of cellular events that can ultimately lead to toxic liver damage (Srivastava et al., 2010).

Hepatotoxicity is a negative effect to liver cells caused by chemical agents (Srivastava et al., 2010). Excess NAPQI can be a hepatotoxic agent. Hepatotoxic conditions due to increased NAPQI can put stress on liver cells and increase the potential for damage. Harmful events due to APAP overdose include the reduction of a liver cell's ability to make ATP energy (Hinson, Roberts, and James, 2010). ATP is needed to drive normal cellular functions. Hepatocyte necrosis can result.

APAP is widely studied as a drug that if taken in large enough quantities predictably results in liver damage (Gunawan & Kaplowitz, 2007; Larson, 2007). APAP hepatotoxicity causes most

cases of acute liver failure, due to drug-induced liver disease. Research implies that potential APAP hepatotoxicity is normally prevented by the hepatocyte's ability to warn other cells of the danger by activation of certain genes within the cells (Coople et al., 2008). Expression of these genes results in cellular defense against the reactive APAP metabolites. Martin-Murphy, Hold and Ju (2009) suggest that these warning signals are measurable indicators of how APAP is affecting the body and may give a potential target for overdose treatment.

How much APAP is too much? The current FDA recommended maximum dose of APAP is 4 grams per day (Tylenol®, 1999). Depending on the health status of the individual, dosage in excess of this maximum may be difficult for the body to safely metabolize. A panel of the FDA has recommended that APAP maximum dosages be lowered and those medications that combine APAP and other active ingredients, such as narcotics or cold medications should be limited or removed from the market (Food & Drug Administration, 2009). These recommendations are currently pending approval. Compromises are being determined so that overdoses can be prevented and those who need APAP can still have access to it.

Some startling statistics regarding APAP usage were identified in a study by Stumpf et al. (2007). Patients do not always know all the facts about their medication or the amount of active ingredient it contains. Only 2% of the patients surveyed could identify how much APAP is a maximum dose while 15% were able to tell which prescription medications also contained acetaminophen as a secondary active ingredient. The majority of patients were unaware that their liver may be damaged if they took too much. Based on those results, patients seem to be at great risk of unintentionally overusing APAP simply due to lack of knowledge about what they are taking. Studies such as this support the FDA panel recommendations to lower APAP dosage (Food & Drug Administration, 2009). Further education of those patients may help prevent APAP misuse.

There is an amount of APAP that will cause ill effects. An extensive medical literature search by Dart and Bailey (2007) determined that the current recommended maximum amount does not typically cause hepatic failure and death. In their estimation, most patients have trouble identifying how much APAP they have taken, thus they may mistakenly consume more than the recommended dose. As a result, patients ended up with liver damage. The current FDA usage guidelines may be sufficient if there is greater understanding of how much APAP should be used in each situation.

The risk of APAP overdose can be minimized but when too much has already been taken, what can be done? Larson (2007) describes 3 factors that may minimize the development of tragic results. These factors are: quick identification of the APAP overdose situation; appropriate response

of healthcare providers to the patient's urgent condition; and prompt treatment to halt the spread of damage before it becomes irreversible. Treatment for APAP overdose involves the administration of N-acetylcysteine immediately after the overdose (Tylenol®, 1999). Therefore, it is important to know when and how much APAP has been taken. Instilling a sense of urgency in healthcare providers to respond quickly with overdose treatment is critical.

Healthcare providers could certainly benefit from learning more about how APAP works but what exactly is important for them to know? Gunawan and Kaplowitz (2007) assert that "The understanding of the mechanism of drug-induced liver injury is of great importance and may lead to prevention and better treatments". This statement highlights the potential benefits of increasing understanding of APAP. The same conclusion was made by Martin-Murphy, Holt, and Ju (2009) as "Elucidation of the underlying mechanism(s) is necessary for identifying predisposing factors and developing strategies in the treatment and prevention of (drug-induced liver injury) DILI". In addition, healthcare providers could benefit from further instruction on APAP usage. Larson et al. (2005) confirmed that "Education of patients, physicians, and pharmacies to limit high-risk use settings is recommended". These statements clearly support increasing awareness in many areas so as to prevent APAP overdose.

New learning tools, such as medical animation, could be used for education. McGill (2008) provided examples of researchers who are combining scientific information with the latest in animation techniques to create medical animations. Many studies have looked at how medical animations are being used as learning tools for cellular and molecular topics. One such study by McClean et al. (2005) examined students who were learning about DNA and RNA. The students who had viewed molecular animations were found to retain more knowledge than those who had access only to traditional methods of learning. The majority of subjects also stated that they preferred learning with the provided animation. Extending the use of medical animation depends on the general acceptance of animations as teaching tools. An increase in the use of 3D medical animations has the consequence of increasing development of technology used in animation.

IV. Project Methods

The project involved the planning and creation of a medical animation on the metabolization of APAP and the associated risk of hepatotoxic liver damage. The steps of project development have been documented including background research, software usage and visual examples of the final animation. The methods used followed a professional medical animation workflow and were adjusted as project work progressed and better options were found. As a result, this project is not a step-by-step tutorial on medical animation creation but rather a guide to what was done during this project. There are many ways to carry out each step depending on the needs of the project and the resources available. The major project steps were pre-production, design, production and post-production.

Project Pre-Production

Pre-production laid the groundwork for subsequent aspects of the project. Because the project emphasized scientifically accurate content, these steps were significantly important and accounted for much of the project timeline. Content development, script development, and storyboarding were done during pre-production.

Content Development

The scope of how much content was to be covered by the animation was decided upon early in the project. Without adequate planning, the project could have quickly become too expansive to show in a short animation format. Identifying the level of detail and amount of content to be covered helped form a story. Content discussed in the literature review was distilled into what could be presented in a few minutes. The most valuable source of content information came directly from the Tylenol® Professional Product Information (PPI) document. What is known about the APAP MOA and metabolism was summarized in the PPI. The names and percentage amounts of the APAP metabolites were also listed. As these and other details were found, they were added to the science reference deck (Appendix A.). The science reference deck contained additional details that were incorporated into model design and animation sequence of action. This deck organized information such as size, alternate names and word pronunciation of story elements.

Molecules and the proteins involved in the story were the main characters for the animation. References for these characters were gathered from the Protein Data Bank (PDB) (http://www.rcsb.org) and PubChem (http://pubchem.ncbi.nlm.nih.gov/). A reference number from these sources identified each structure. These numbers were noted in the science reference

deck. 3D structures of the enzymes cytochrome P450 and glutathione transferase were located on the PDB. Other versions of these proteins were available on the PDB but many were of different sub-types and not relevant to the story. The 3D PDB files were downloaded as a .pdb file type. APAP, NAPQI, GSH, NAC, and the 5 metabolites of APAP were located on PubChem. The 3D PubChem structures were downloaded as .sdf files. All 3D structure files were saved for later use in development of 3D models.

The hepatocyte was identified as the main cellular character. In the liver, hepatocytes are grouped in lobules so the lobule was added to the list of models to be created. The liver and stomach as entire organs were needed as story elements and for an environment. A representation of a bottle of Tylenol® and tablets of APAP were also to be modeled. Image research found examples of how all these characters have been represented. The source or location of reference images was noted for future reference.

Script Development

Before beginning the script, a bullet point outline was developed. This outline contained the names of all characters and a basic layout of the story flow. The script (Appendix B.) consisted of columns covering various details of the animation. One column was animation notes, a description of the actions and movements of characters in that portion or scene of animation. The accompanying narration or voice over (VO) was contained in another column. At about 100 words per minute, the VO was used to help with timing and provided an estimated total animation length. The final script was 260 words in length, indicating that the animation would be approximately 2.5-minutes long. This length was determined to be appropriate for the project's scope yet still contained enough content to create an interesting animation. The VO verbally reinforced important content points demonstrated with animation. An additional column of the script listed onscreen text to be shown during each story section. The VO was then recorded as an audio file, details of which are provided in the sound editing section.

Storyboarding

Storyboards, a basic visualization of the story, helped with pacing and gave an approximation of the anticipated visual flow of the animation. Very rough thumbnail sketches were done in conjunction with script development in order to keep the visuals in line with the story. After the script was "locked", final storyboard images were drawn in pencil and scanned for modification in the computer. The images were drawn in a widescreen format to match the desired aspect ratio for the animation. An individual storyboard image was created for approximately every sentence of the

script. Arrows were added to some images to indicate the direction of motion for the characters. Labels of onscreen text were added to the images. The images, along with the animation notes and VO, were assembled into a deck so as to be referred to as production progressed.

Animated storyboards, called a 2D animatic, combined the static storyboards images with the VO audio. Apple iMovie was chosen for this purpose due to ease of adding or updating elements. Image and audio files were imported into iMovie and dragged to the timeline where they could be rearranged. The waveform of the VO audio file was visible in the timeline and the display length of the images could be adjusted to match the timing of the VO. A movie file of the animated storyboards was made. Viewing the 2D animatic indicated a few content and story flow problems that were addressed with script rewrites and storyboard image revisions. These changes were easier to make at this stage than after production had begun.

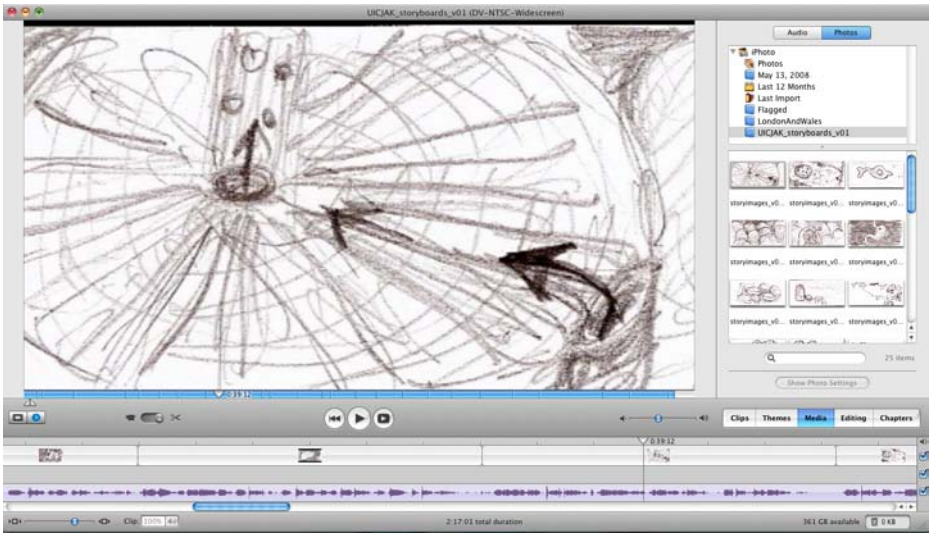


Figure x. Creation of animated storyboards in Apple iMovie.

Project Design

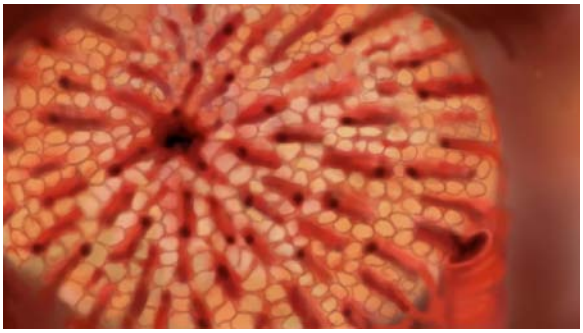
The design steps of the project included visual aspects that were included in the final animation. From mood boards and concept art to modeling, textures and lighting, these steps brought the project into 3D by providing the characters and scenes with a specific look.

Concept Art

Concept art consisted of mood boards and style comps to create a look for the animation. Mood boards featured various images and photos inspiring a color palette and the environmental feeling for the scenes and characters. A total of 9 mood boards were created. Reference photographs were

found of the Tylenol® bottle, tablets, and liver tissue. Some mood boards were exclusively for look and feel. Mood boards contained images of honeycombs, chalky candies, gummy bears, and APAP metabolic pathway diagrams. These mood boards were compiled into a deck with written descriptions of how the images related to the anticipated scenes and characters.

Style comps were pieces of art created specifically for the animation that illustrated the environments or characters in the established look and feel of the mood boards. A storyboard sketch was used as the base drawing. 2D software was used to paint color on the image in layers that could further be edited. Style comps were created for 3 major scenes of the animation: the liver lobule, the hepatocyte, and the intracellular molecular environment. Reference images used for the liver lobule consisted of scanning electron micrographs (SEM) of hepatocytes and photos of the arrangement of honeycombs. The concept art for the individual hepatocyte was to show internal organelles and nuclei. The coloring of these elements was purely artistic. Bright colors were used to make elements discernable. The content development step had identified the smooth endoplasmic reticulum as the main site of APAP metabolism. The coloring the background for the molecular environment reflected the coloring of the smooth endoplasmic reticulum. This was done to help orientate the viewer as to where they were during animation scene changes.



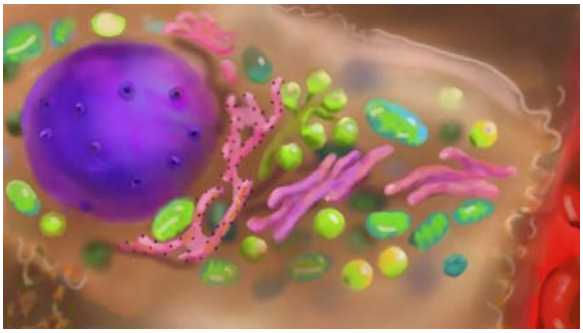


Figure x. Concept Art – Hepatocyte

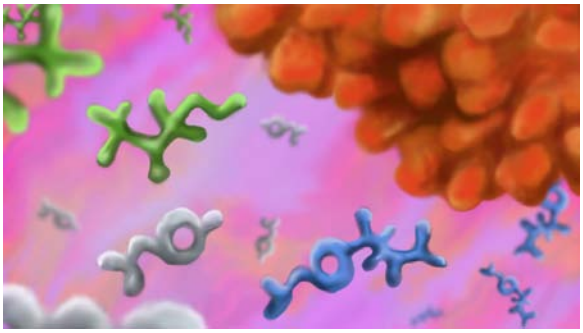
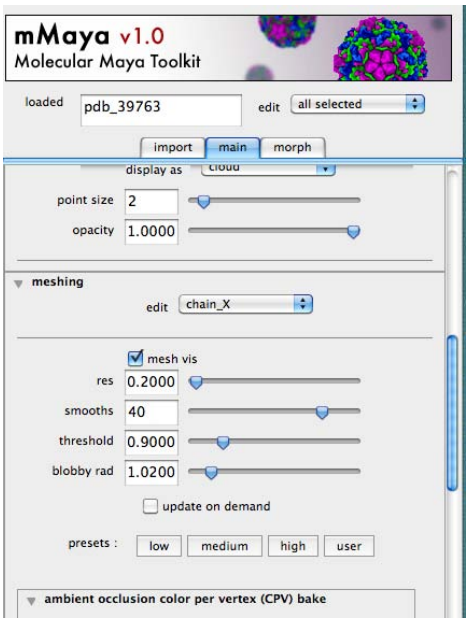


Figure x. Concept Art - Intracellular Environment

3D Modeling

Characters, illustrated in storyboards and style comps, were developed into 3D models. A preliminary model book was assembled with model sketches and default renders of the individual

models that were to be created or modified from existing models. The PDB and PubChem numbers were used for reference of the original 3D file to be used during modeling.



Molecule files in a PDB file format could be directly imported into Maya® with the mMaya plug-in. Files from PubChem first needed to be converted into PDB files. The UCSF Chimera molecular visualization program (http://www.cgl.ucsf.edu/chimera/) was used to open the PubChem .sdf files and save them in a PDB file type that could be used with mMaya. Once the files were imported into Maya, nParticle settings for each model needed individual adjustments to the resolution, smoothness, threshold, and blobby radius of the mesh surface. When the desired look was achieved, the nParticle surface was converted into a polygon surface and the model was saved as an .obj file. More information on the use of mMaya is contained in tutorials on the Molecular Movies toolkit help page (http://www.molecularmovies.com/toolkit/index.html).

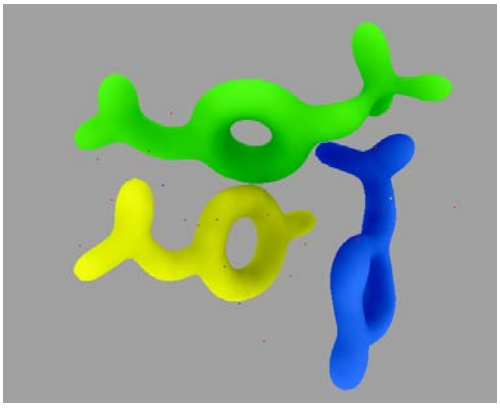


Figure x. Molecules – APAP and Metabolites

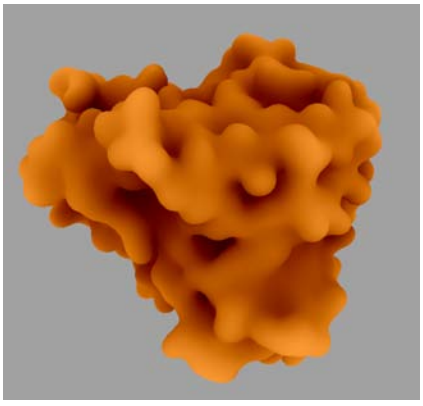
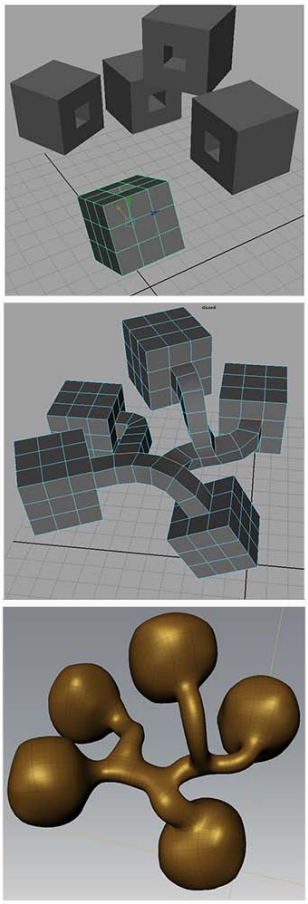


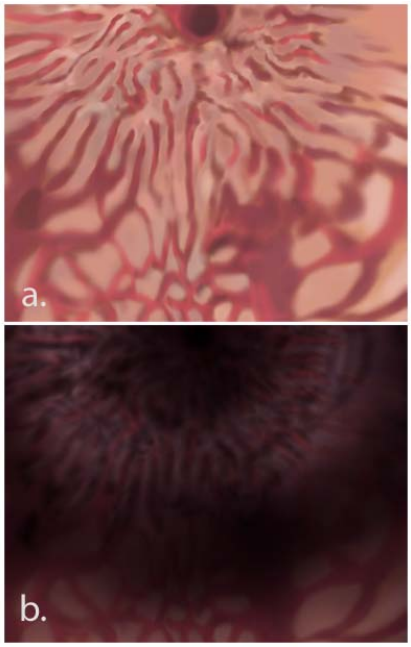
Figure x. Protein - Cytochrome P450 Enzyme

Other models were specifically created for this animation including an APAP bottle, APAP tablets, liver lobule, and a hepatocyte with intracellular structures. Various modeling techniques were used for these models. The profile of the APAP bottle was drawn with a spline tool and revolved to create a 3D shape. The tablets were modified from a cylinder shape by adding a groove down the center. The liver lobule, hepatocyte, and intracellular structures started as basic polygon shapes in Maya®. These shapes were exported as an .obj file, smoothed and modeled with Mudbox®, and then imported back into Maya® as a new .obj file. Figure x. shows an example of this process with the construction of the intracellular Golgi apparatus. Mudbox® was particularly useful in sculpting surface detail of the liver lobule and the exterior hepatocyte surface.



The liver and stomach models were modified from purchased stock 3D assets. These stock models were also sculpted slightly in Mudbox® in order to blend with the style of the newly created models.

Texture and Lighting



The 3D elements were textured to resemble the concept art. Texture development involved trial and error of creating and correctly placing surface textures, called shaders, on the models. Mudbox® was used to paint the 3D surface of the liver lobule model. The initial painted color map was exported from Mudbox® as a tiff image. Photoshop® was used to add additional detail to the healthy lobule and then to paint a damaged version. These images were saved as png files for use in the shader network in Maya®. The surface map was placed on the lobule model as a color shader with a slightly glossy Blinn surface. Figure

X shows examples from the final painted maps for the healthy and damaged lobule.

The APAP tablets, molecules, and proteins were surfaced with basic Lambert shaders that had a dull surface appearance. APAP was colored white to resemble the texture of the initial tablet, while NAPQI was colored grey to indicate that it was very structurally similar to APAP. Bright colors were chosen for the remaining metabolite shaders to match the concept art and to clearly distinguish one from another. The layering of render passes was tested and it was decided that an ambient occlusion layer would help give the molecules a chalky surface texture desired.

The APAP bottle and cap were shaded with a basic Blinn shader. The bottle needed a label applied and a cylindrical UV map was used to correctly orientate the label image. Figure x demonstrates the proces needed for label placement. The ground plain under the bottle and tablets used an image of a paper cloth that was repeated to cover the entire surface.

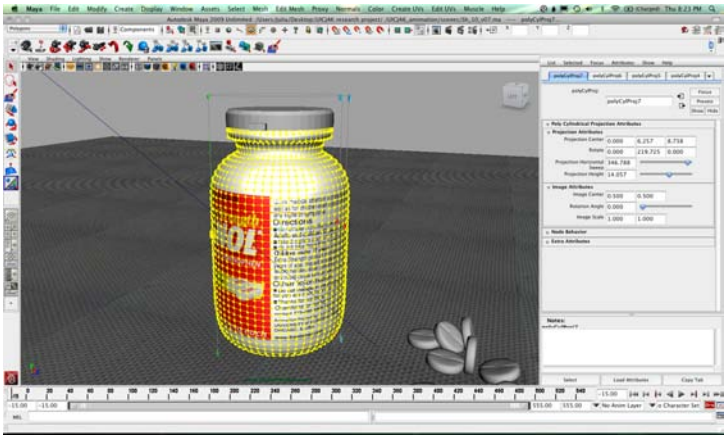


Figure x. Placing a UV map for the bottle label.

Preliminary lighting of characters and scenes was tested as scenes were set up. The APAP bottle scene was determined to need a 3-point light set-up with shadows enabled for the realistic look that was desired. The other scenes of the animation were to be less realistic and the lighting was kept more basic. An ambient light source and an additional directional light were sufficient for most scenes. Shadows for the molecules and other models were not directly created with 3D lighting. Rendering of those scenes was to be done with separate ambient occlusion layer to be applied during compositing, which would give the appearance of shadows.

Project Production

The production stage of the project turned the prepared visual elements into 3D animation. A spreadsheet shot list was created to track elements to be added to each scene and to refine the shot timing of the animation. The shot list divided the entire animation into individual camera shots according to the VO and description of what was to be animated. The length of shots in the 2D animatic provided the number of frames required for the animation. This was done by multiplying

the time in seconds by 30 to give the number of frames per second. 15 frames were also added to both ends of the shot to allow for transitions between scenes. Shots were assigned a number set a standard format for the naming convention of all files associated with each shot.

3D Animatic

The 3D animatic was created by populating each scene with 3D elements according to their arrangement in the storyboard images. The animated storyboards were used as a timing reference for fine-tuning the motion of the characters. The high-resolution 3D models in some shots were temporarily substituted with low-resolution 3D models to save on computer processing and preview rendering time. Default gray colors were applied to the models at this stage. The framing of the scenes was blocked out using cameras in Maya®. The scenes were previewed as Maya® playblast animations. The playblast animations were stored as movie files and were composited together with the VO audio track to create the 3D animatic for review. After viewing the 3D animatic, any necessary timing and motion changes were incorporated to better align the visuals to the VO.

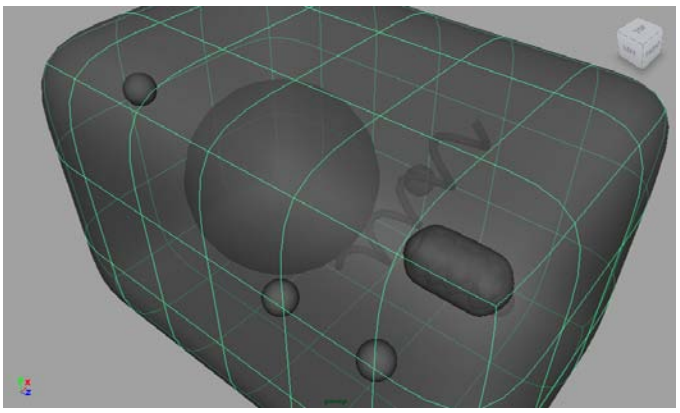


Figure x. Low-resolution hepatocyte render.

Animation

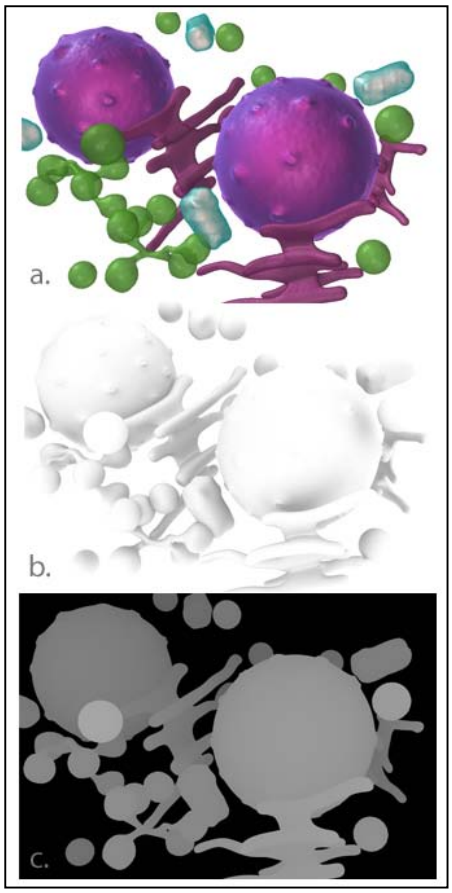
Movements of characters, cameras and scene elements were refined. Low-resolution models were replaced with the final high-resolution files. The accuracy of the characters was marginally adjusted in order to tell a coherent and relevant story. The relative size and speed of character movement and the density of the cellular and molecular environments are examples of alterations required to increase clarity. Once the animation of all individual 3D scenes was "locked" they were colored, shaded and lit with the lighting that had been developed during the design stage. The concept art was used as a guide for the look and feel. Render tests were done to ensure scenes were rendering properly and looked as expected. As test renders were made, they were added to the compositing file in order to view the animation as a rough cut. Scenes were then prepared for final rendering by deleting excess models and checking that the render settings were correct. Removal of excess geometry from the scene shortened render time. The Maya® render settings were adjusted so that only the correct frame range of the scene would be rendered by the desired camera.

Project Post-Production

Post-production included the finals steps of the project. Rendering and compositing turned the grey shapes from the 3D animatic into a final animation. The file was rendered from the compositing program and converted into various file formats so as to be playable on different computer systems.

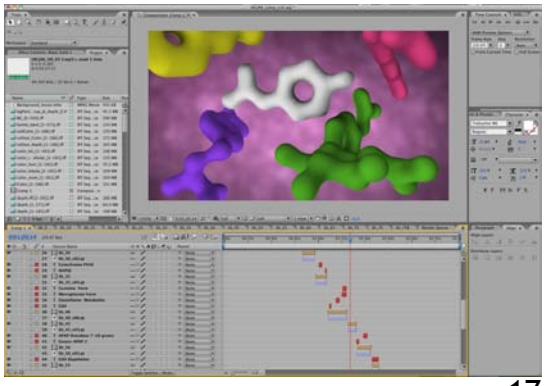
Rendering

Each frame of the animation was rendered as a separate image in order to prevent loss of work if the entire sequence did not render correctly. Rendering was also done in layers so that post effects could be applied during compositing. The native Maya® file format of .iff was used as image file extension format. Maya® was set to automatically render a batch of frames while adding the shot number, layer name, and frame number to each file. The main layers for each shot were a color shader layer (Figure x. – Image a.), ambient occlusion pass (Figure x. – Image b.), and a depth pass (Figure x. – Image c.). The depth pass render settings had to be calibrated individually for each shot based on the distance of the models in the scene from the camera. A static image of a sphere with a surface bump texture was rendered for use as a mottled background in the intracellular scenes. The render time of an individual frame for most render layers was a few seconds. The ambient occlusion pass had a render time of 1-3 minutes per frame and was rendered last in case any animation changes were needed. Rendering all layers for each shot took many hours so rendering was planned for night or when the computer was otherwise not in use.



Compositing

Compositing brought the various individually rendered image files together. The After Effects® composite used for the 3D Animatic and Rough Cuts was again used as a base for the final comp. Individual image frames were imported into After Effects® and pre-composited into the appropriate shots. Simple motion



graphic labels were added. The depth pass was used in an adjustment layer with a lens blur effect so that the background and extreme foreground objects could appear out of focus. The ambient occlusion layer was set to multiply in order to give the shadow effect to the models. A solid black layer with a feathered subtraction mask was added above the background layer to darken the edges and vignette the scene. Other post-effects such as glows were added to slightly highlight the molecules.

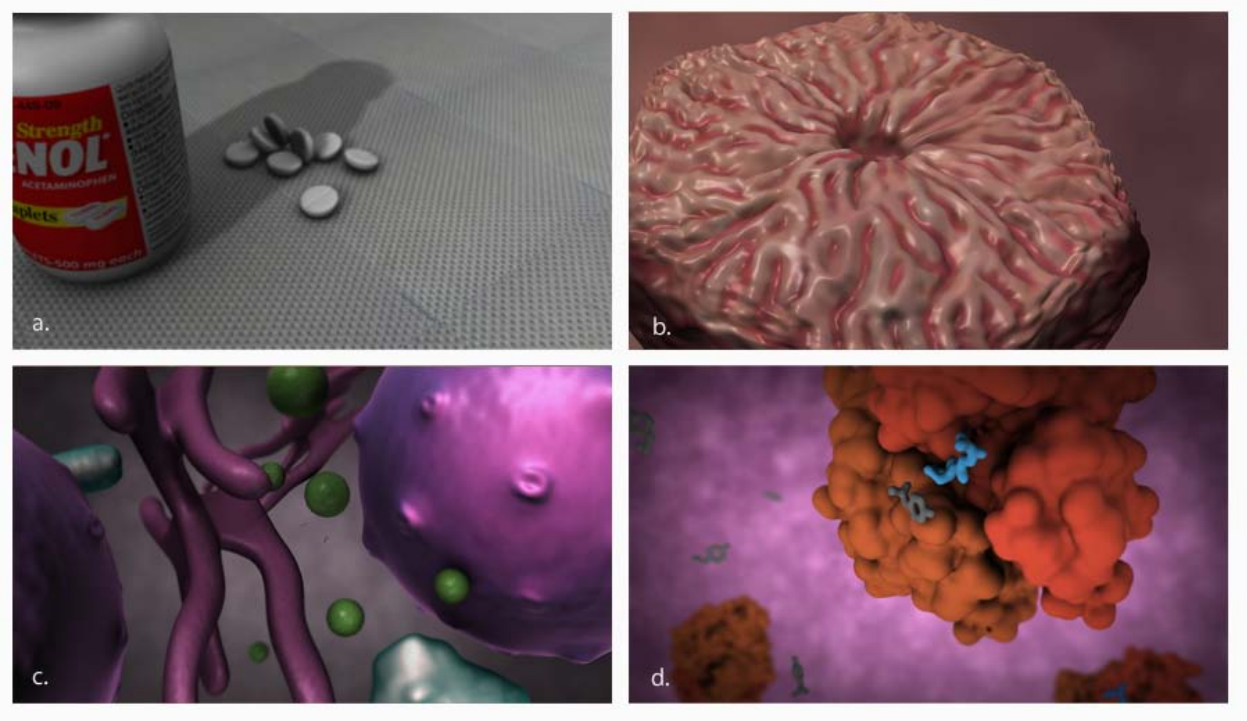


Figure x. Composited screenshots of the final animation.
a. APAP bottle and tablets. b. Normal hepatocyte. c. Individual hepatocyte. d. Intracellular molecules and protein.

Sound Editing

The VO script was recorded with an external microphone connected to the computer. Garage Band was set to record a voice audio file, such as for a podcast. The VO script was recorded in short segments according to the shot list. The segments were previewed by playing back and adjustments to the pacing and pronunciation were made until each track sounded consistent. The individual segments of audio were aligned in the Garage Band timeline and saved as one audio file. This audio file was imported into the various composites. A basic music track from the Garage Band library was selected for background music. The music track was not as long as the animation so it was copied and repeated several times to provide music for the entire animation.

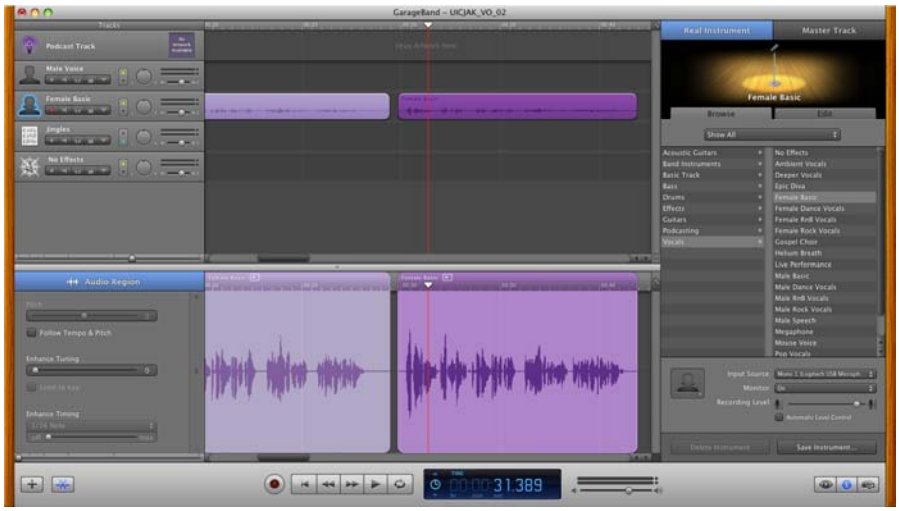


Figure x. VO recording process in Apple Garage Band.

Summary of Steps

PRE-PRODUCTION	DESIGN	PRODUCTION	POST-PRODUCTION
Content Development	Mood Boards	Shot List	Animation Full Render
Science Reference Deck	Model Sketches	3D Scene Set-Up	Motion Graphic Labels
Script (VO and Action)	Concept Art	3D Animatic	Visual Post Effects
Storyboards	3D Modeling	3D Character Animation	VO Recording and Music
Voiceover Recording	Textures and Lighting	3D Camera Animation	Final Composite Rendering
Animated Storyboards		3D Effects	
		Rough Cuts	

Table 1. Summary of project steps

Project Management

The overall project development was helped by the use of organization and project management software. A program called Papers (http://mekentosj.com/papers/) was used during the literature review and content development to organize reference articles and PDFs. The search feature of Papers also lead to the discovery of additional content assets. Zotero (http://www.zotero.org/) was used to track and save the location of online resources and images. A summary of the references was uploaded to RefWorks (http://refworks.com/) in order to apply inline citations and populate the reference page in the appropriate format. OpenProj (http://openproj.org/), an open source project management tool, was used to create and update the project timeline. This timeline was set to show how upcoming steps were reliant on the completion of previous steps. Background research and project organization benefited from the use of these tools.

A project website was created as a place to share the project and animation development steps with project advisors and other reviewers. The current version of the decks of storyboards, mood boards, concept art, and script were added to the project website. Videos of the animated storyboards, 3D animatic, and rough cuts were uploaded to YouTube (http://www.youtube.com/). Web links to the YouTube video were embedded in the project website so as to provide one location for all project material.

Hardware & Software Resources

This is a summary of the specific hardware and software used during the project. A MacBook Pro computer (Apple Inc., Cupertino, CA) was used for the majority of the work. Various 2D artwork elements, such as storyboards and concept art, were hand-drawn, digitally scanned then modified in SketchBook Pro 2009 (Autodesk® Inc., San Rafael, CA) and/or Adobe® Photoshop (Adobe Systems, San Jose, CA). The voice over script was digitally recorded with an external microphone and Garage Band (Apple Inc., Cupertino, CA). Animated storyboards were created with iMovie (Apple Inc., Cupertino, CA). The primary 3D software was Maya® 2009 (Autodesk® Inc., San Rafael, CA). Maya® was used for all the 3D aspects of the project including modeling, animatic, animation and certain effects. Molecular structures were imported with the mMaya plug-in (http://www.molecularmovies.com/toolkit/index.html). Mudbox® 2009 (AutoDesk® Inc., San Rafael, CA) was used for sculpting of certain 3D models. Adobe® After Effects® (Adobe Systems, San Jose, CA) was used to composite the individual files into the final animation. Certain post-effects and visual treatments also were done in Adobe® After Effects®.

Timeline

		Name	Duration	Start	Finish	Predeces...
1		**⊟Julia's Research Project**	**155 days**	**12/21/0...**	**7/23/10 5:00 PM**	
2		**⊟Pre-Production**	**100 days**	**12/21/0...**	**5/7/10 5:00 PM**	
3	✔	Proposal Draft	16 days	12/21/09...	1/11/10 5:00 PM	
4	✔	Proposal Draft Advisor Review 1	5 days	1/11/10...	1/15/10 5:00 PM	3
5	✔	Proposal Revisions 1	19.125...	1/12/10...	2/8/10 9:00 AM	3
6	✔	Proposal Advisor Review 2	2 days	2/9/10 8:...	2/10/10 5:00 PM	5
7	✔	Proposal Revisions 2	18 days	2/11/10...	3/8/10 5:00 PM	6
8	✔	Proposal Advisor Final Review	2 days	3/9/10 8:...	3/10/10 5:00 PM	7
9	✔	Proposal Final Revisions	1.25 days	3/11/10...	3/12/10 10:00 AM	8
10		Proposal Full Committee Review	33.75 d...	3/12/10...	4/28/10 5:00 PM	9
11	✔	Script Development	11.75 d...	3/12/10...	3/29/10 5:00 PM	9
15		Science Reference Deck	36.75 d...	3/12/10...	5/3/10 5:00 PM	7
12	✔	Script Revisions	18 days	3/30/10...	4/22/10 5:00 PM	11
13	✔	Mood Boards	5 days	4/12/10...	4/16/10 5:00 PM	12
14	✔	Storyboard Development	3 days	4/23/10...	4/27/10 5:00 PM	12
16	✔	Advisor Storyboard & Script Revi...	2 days	4/28/10...	4/29/10 5:00 PM	14
17	✔	Storyboard revisions	5 days	4/28/10...	5/4/10 5:00 PM	14
18	✔	Voiceover Recording	1 day	5/7/10 8:...	5/7/10 5:00 PM	17
19		**⊟Design**	**35.5 days**	**4/26/10...**	**6/14/10 5:00 PM**	
20	✔	Model Sketches	6 days	4/26/10...	5/4/10 1:00 PM	14
26	✔	Animated Storyboards	11 days	4/28/10...	5/12/10 5:00 PM	14
23		Models Development	14 days	4/28/10...	5/18/10 1:00 PM	14
24	✔	Model Book, Storyboards, Script...	5 days	5/5/10 8:...	5/11/10 5:00 PM	17;20;26
21	✔	Style Comp Development	17.5 days	5/10/10...	6/2/10 5:00 PM	20;13
22		Style Comp Advisor Review	2 days	6/3/10 8:...	6/4/10 5:00 PM	21
25		Textures Development	8 days	6/3/10 8:...	6/14/10 5:00 PM	21;23
27		**⊟PRODUCTION**	**33 days**	**5/10/10...**	**6/23/10 5:00 PM**	
28	✔	Shot List	3 days	5/10/10...	5/12/10 5:00 PM	17
29	✔	3D Scene Set Up	12 days	5/20/10...	6/4/10 5:00 PM	26;28
30	✔	3D Animatic	12 days	5/20/10...	6/4/10 5:00 PM	29
31	✔	3D Animatic Advisor Review	3 days	6/7/10 8:...	6/9/10 5:00 PM	30
32		Animation Finalization	10 days	6/7/10 8:...	6/18/10 5:00 PM	30
33		Rough Cut 1 (Some renders)	5 days	6/7/10 8:...	6/11/10 5:00 PM	29;30
34		Rough Cut 1 Advisor Review	3 days	6/14/10...	6/16/10 5:00 PM	33
35		Lighting 1st. Pass and Testing	5 days	6/14/10...	6/18/10 5:00 PM	33
36		3D effects development	5 days	6/14/10...	6/18/10 5:00 PM	33
37		Render Final – Full Resolution	3 days	6/21/10...	6/23/10 5:00 PM	36
38		**⊟POST-PRODUCTION**	**30 days**	**6/14/10...**	**7/23/10 5:00 PM**	
39		Motion Graphics	10 days	6/14/10...	6/25/10 5:00 PM	33
40		Post Visual Effects Development	5 days	6/14/10...	6/18/10 5:00 PM	33
41		Music & Sound Effects Developm...	5 days	6/14/10...	6/18/10 5:00 PM	33
45		Final Paper	10 days	6/14/10...	6/25/10 5:00 PM	33
42		Render out of After Effects	3 days	6/21/10...	6/23/10 5:00 PM	40
46		Prepare final presentation	10 days	6/28/10...	7/9/10 5:00 PM	39;40;4...
44		Project Advisor Sign Off	5 days	6/30/10...	7/6/10 5:00 PM	37;45
43		Final presentation	2 days	7/22/10...	7/23/10 5:00 PM	44

Table 2. Timeline created in OpenProj.

Discussion

The development of an animation on the APAP metabolic pathway and production of byproduct metabolites was the goal of this project. An animation was created that incorporated concepts of APAP metabolism and related liver damage. The type and amount of content shown in the animation was designed with the needs of an audience of healthcare professionals in mind. Extensive background research provided the necessary level of detail for this audience. A main source of project content was the Tylenol® Professional Product Information (PPI) which describes the absorption and breakdown of APAP into metabolites. Another focus of the story was the current FDA recommendations for overdose prevention. It is hoped that this animation could be used for education by providing the viewer with a vibrant visual representation of the science of APAP metabolism and hepatotoxicity.

The metabolic pathways and sites of action identified were used as locations in the animation. The scenes of the animation were planned to gradually bring the viewer from a gross level to a molecular level of magnification, and from the easily recognizable to the unknown. The pill bottle and tablets were introduced into the animation to give a familiar point of reference for the beginning of the animation. APAP absorption and delivery to the liver was shown with a depiction of the entire stomach and liver. The focus then shifted to a more microscopic level by showing the intermediate liver lobule followed by an individual hepatocyte. The VO was used to indicate the site of APAP metabolism as the smooth endoplasmic reticulum, while the animation cut visually to the nanometer level of magnification where molecules and enzymatic proteins are located. There, the animation dynamically presented the APAP metabolic pathway as interaction between 3D models as opposed to the typical diagrammatic representation of these pathways as shown in many references. Content was presented in a scientifically accurate manner with certain liberties taken for storytelling purposes. By bringing the story to life in this fashion, the animation provides an engaging display of APAP metabolism.

The steps used to plan and create the animation were adapted from the workflow for similar animations created by professional medical media companies. Project roles normally would be broken down so as to be completed by a team of people. For this project, all roles were consolidated so that one person could complete an animation with simplified content and visuals. Due to this limited project scope, some steps were not fully completed and the animation is not as polished as a professional piece. For example, only relatively basic shaders and lighting were used and final

renders were not as complex. Even with these simplifications, the animation could potentially be useful to the viewer.

Since the project was an exploration of all the steps involved in creating a medical animation project, many techniques had to be learned. Maya® was chosen as the 3D software in order to gain more working experience with Maya® and so that the mMaya plug-in could be used. In general, using Maya® was a challenge since its workflow differs from other 3D software. Some areas of Maya®, such as modeling and key frame animation, were very similar to other programs. While shader networks and lighting were a more complex challenge to learn. Another major step of the animation project was determining the story to be shown. Even with a full-length movie, it would not be possible to show every detail of the story. Therefore this project also demonstrated how the scientific content could be consolidated yet still make important points.

Pitfalls were encountered during project development, but these did not greatly interfere with completion of the animation. Since only one person performed all steps, there were some areas that required simplification due to lack of expertise. Another major hindrance was the limited amount of resources and time available to complete the project. It is difficult to check one's own work or to know if something would make sense to others. Even with those problems, the project was completed and the information gained during the process was extremely beneficial. How the process could have been made easier can be drawn from these results. More experience with the various software and techniques would have helped. Additional machines could be used to decrease render time and allow for more elaborate effects. The methods section described the key elements involved in this project's animation process. If another individual wished to create an animation with a similar project scope, those methods should be adapted based on that the individual's skills and goals for their project.

Expanding the use of medical animations may lead to further improvements in technologies specifically designed for their creation. One example of this animation technology is mMaya, a Maya® plug-in for molecular visualization (http://www.molecularmovies.com/toolkit/index.html). The mMaya plug-in provides the ability to directly import molecular structures into Maya®. Before mMaya was developed, more steps were required to import these structural components into a 3D program. This project used mMaya to accurately show molecules of APAP, its metabolites, and various metabolic enzymes. Further improvements in creating medical animations depend on the development and usage of these new tools.

Conclusion

The project successfully designed and created an animation on the metabolism and hepatotoxcity of acetaminophen. Steps of project development were based on a professional medical animation workflow. These steps were consolidated to fit the project scope and the abilities of the researcher. A Maya® plug-in called mMaya was used create new shapes for the molecular characters. The relevance of mMaya is that it gives a new way to design and create medical animations. All project steps of design and creation of the animation were documented. Another researcher could potentially use this project's methods as a starting place to create a similar or even a more elaborate medical animation. As the topic of the animation, APAP metabolism provided an interesting story with many scenarios that could be visualized. The anticipated needs of an audience of health care providers were considered. FDA guidelines and maximum APAP dosage recommendations were used to create a story that educates on what happens if too much APAP is taken. It is hoped that user testing would prove this animation to be a useful learning tool for a target audience of healthcare professionals.

Technical and Creative Thanks

Support in creating this project was provided by individuals from Eveo inc. (www.eveo.com) a San Francisco, CA based medical media agency. People offering assistance included; storyboard, 3D and motion graphic artists, medical writers, project producers, and art directors. They provided technical, creative, and project management advice as needed. Special Thanks to Baron Gumban, Dario Lopez, Erich Rigling, Henry Chen, Kevin Ang, and everybody else who supported the project efforts along the way.

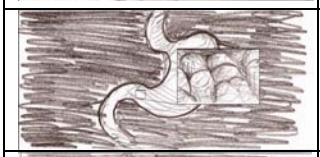
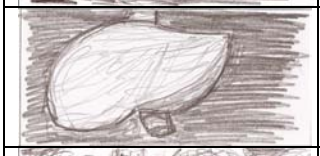
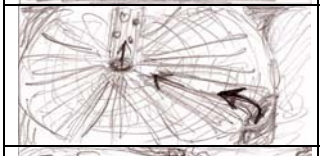
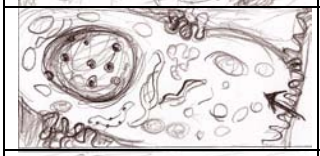
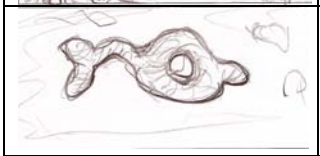
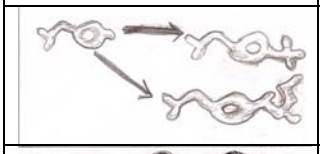
Appendix A. – Science Reference Deck

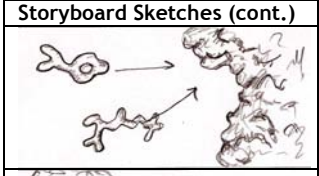
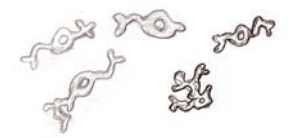
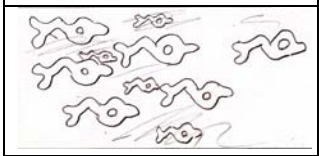
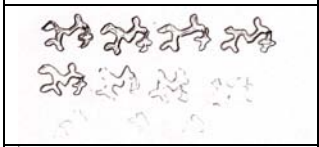
This science reference deck was created during content development and contains additional science information and location of the sources of molecular data used during animation development.

Character Name (molecular level)	PDB/ PubChem	Also known as/ Type	Size/Weight	Pronunciation	Notes
Acetaminophen	CID_1983	APAP, paracetamol, Tylenol®	151.17 g/mol	acet·amin·o·phen ə-ˌsē-tə-ˈmi-nə-fən, ˌa-sə-tə-	
Acetaminophen cysteine	CID_83997	APAP metabolite	254.31 g/mol	cys·teine ˈsis-tə-ˌēn	
Acetaminophen glucuronide	CID 83944	APAP metabolite	327.29 g/mol	gluc·uro·nide glü-ˈkyur-ə-ˌnīd	
Acetaminophen sulfate	CID 83939	APAP metabolite	231.23 g/mol	sul·fate ˈsəl-ˌfāt	
Acetaminophen glutathione	CID_83998	APAP metabolite	327.29 g/mol	glu·ta·thi·one ˌglüt-ə-ˈthī-ˌōn	
Acetaminophen mercapturate	CID_539698	APAP metabolite	312.34 g/mol	mer-cap-TUR-ate	
NAC	CID_12035	N-acetylcysteine, overdose antidote	163.19 g/mol	ace·tyl·cys·te·ine ə-ˌsēt-ˈl-ˈsis-tə-ˌēn	
Cytochrome P450	2J0D	Enzyme, CYP2E1, CYP1A2, CYP3A4	Length [Å] a = 67.25 b = 210.71 c = 161.25	cy·to·chrome ˈsīt-ə-ˌkrōm	
Glutathione	CID_124886	GSH, tripeptide, cofactor	307.32 g/mol	glu·ta·thi·one ˌglüt-ə-ˈthī-ˌōn	
Glutathione transferase	1EOH	Enzyme, protein, polypeptide	Length [Å] a = 82.97 b = 84.02 c = 236.97	glu·ta·thi·one ˌglüt-ə-ˈthī-ˌōn	
NAPQI	CID_39763	N-acetyl-p-benzoquinoneimine, APAP bioactive metabolite	149.15 g/mol		
COX	1PRH, 4COX	Enzyme, Cyclooxygenase	Length [Å] a = 179.80 b = 133.60 c = 118.40		Not used in the animation

Character Name (cellular/systems level)					
Liver	N/A	Organ			
Hepatocyte	N/A	Cell		he·pa·to·cyte hi-ˈpat-ə-ˌsīt	
Smooth endoplasmic reticulum (ER)	N/A	Organelle		en·do·plas·mic ˌen-də-ˈplaz-mik re·tic·u·lum ri-ˈtik-yə-ləm	
Golgi apparatus	N/A	Organelle			

Appendix B. – Script, Storyboard Sketches, VO, Text on Screen, Animation notes

Storyboard Sketches	VO - Word Count 260	Text on Screen	Animation Notes
	Acetaminophen or APAP is a widely used medication with analgesic and antipyretic properties.	Acetaminophen APAP	Fade in. Shown is a bottle of APAP and tablets of APAP with a neutral background.
	The FDA recommends a maximum dose of 4 grams of APAP per day. Consuming more APAP than recommended can potentially cause severe liver damage.	Maximum APAP dose 4 grams per day	Camera pushes in to show what 4 grams of active ingredient looks like (eight 500mg tablets = 4grams).
	When APAP is taken orally…		Cut to a single APAP tablet moving downward in the stomach. The stomach will be simple and no other stomach contents will be shown. A visual effect shows the tablet dissipating to individual particles representing APAP molecules.
	…it absorbs rapidly in the upper GI tract. APAP enters the bloodstream and is carried to sites of action.		Picture-in-picture is faded on and shows villi surface of GI tract. Picture-in-picture cross fades to show APAP passing though the surface of the villi.
	The liver metabolizes APAP and produces metabolites required for function.	Liver	Stomach fades off and liver fades on.
	Many metabolic pathways occur in the liver to rid excess APAP and other substances from the body.	Liver Lobule	Cut to interior of liver. Healthy hepatocytes are arranged in a symmetrical pattern. Blood cells are flowing along arrow path.
	APAP is metabolized in the hepatocyte's smooth endoplasmic reticulum.	Hepatocyte	Cut to single hepatocyte that nearly fills the screen. Simple cellular contents are shown including; nucleus, endoplasmic reticulum, golgi, mitochondria etc.
	The majority of APAP…	APAP	Cut to a simple intracellular space environment, the same color of the smooth endoplasmic reticulum. The APAP molecule structure is shown. More molecules will be seen in the background throughout all molecular shots.
	…is conjugated by enzymes into inert sulfate and glucuronide metabolites.	APAP Sulfate APAP Glucuronide	Camera move to show scene wider. Labels appear with the 2 metabolites.
	An enzyme, cytochrome P450, converts the remaining APAP…	APAP Cytochrome P450	Cut to focus on Cytochrome P450 as it drifts into view. APAP enters the active site of the enzyme.
	…to NAPQI.	NAPQI	NAPQI exits the enzyme.

Storyboard Sketches (cont.)	VO – (cont.)	Text on Screen	Animation Notes (cont.)
	NAPQI can be combined with GSH…	NAPQI GSH	Cut to another enzyme. Both NAPQI and GSH enter the active site.
	…to create an intermediate glutathione metabolite. Further conjugation results in mercapturate and cysteine forms.	APAP Glutathione APAP Mercapturate APAP Cysteine	APAP Glutathione exit the enzyme. Same arrow and label treatment is used for these 2 end product metabolites.
	Inert metabolic end products are harmlessly eliminated in the urine.		Camera drifts to frame up the end products. These metabolites are shown moving away from center and off frame. Fade to black.
	The situation changes if an excessive amount of APAP enters the system. An overdose of 7-10 grams of APAP in one day…	Excess APAP	Fade in to slightly different (color) cellular environment. More APAP molecules are seen. Some in background convert to the first 2 metabolites. Most convert to NAPQI.
	…quickly exhausts the GSH available for NAPQI deactivation.	APAP overdose 7-10grams	GSH molecules are shown but there are not enough to pair with all the NAPQI.
	NAPQI is toxic to cellular proteins and nucleic acids.	NAPQI	Cut to show single NAPQI.
	Damage to intracellular structures causes irreversible harm to the hepatocyte.		Cut to NAPQI moving away from the area to structures nearby which lose color and look ill.
	Spreading hepatocyte destruction can lead to hepatic failure and patient death.	-Hepatocyte Destruction -Hepatic Failure -Patient Death	Cross fade to liver lobule now with obvious damage. Show 3 bullet points in time to VO. Blow out to white.
	There are ways to avoid such consequences. N-acetylcysteine, called NAC is the antidote for APAP overdose.	NAC	Fade in from white to ambiguous cellular environment. NAC pathway is shown with 3D molecules. Excess NAPQI is in the environment.
	If NAC is given in time, it converts to GSH for deactivation of excess NAPQI before hepatotoxicity can occur.	NAPQI GSH	NAPQI is converted by the enzyme into the metabolite.
	Increasing general awareness of appropriate APAP usage could help prevent overdoses from occurring.	Prevent APAP Overdose	Cut to pill bottle of APAP and tablets. Same scene as the beginning. Screen fades to black. The end!

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