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Breaking down apoptosis: animating programmed cell death in 3D for a pathology curriculum

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ABSTRACT
Contemporary medical education is expected to keep up with the rapidly expanding corpus of medical scientific knowledge to train informed doctors. Swift communication and assimilation of complex concepts are required, yet traditional teaching methods are often suboptimal means to this end. This paper details the making of a concise 3D animation on the apoptotic pathways, designed to improve first-year undergraduate medical students’ grasp of cell signaling. A simplified visual language was adopted to increase the effectiveness and expedite the production of beginner molecular biology animations. Favourable student feedback suggests that the chosen design approach could yield further positive results.

Introduction
The University of Dundee’s MSc Medical Art programme frequently cooperates with the School of Medicine and the Technology and Innovation in Learning Team (TILT) at Ninewells Hospital in Dundee to create e-learning resources for their medical curriculum. A recent such collaboration resulted in the production of a bespoke four-minute 3D animation on apoptosis, or programmed cell death. This paper discusses how the project came about in response to specific pedagogical demands, then details the reasoning behind the design approach and describes the implemented workflow. Finally, it considers students’ responses to the work.

Broadly stated, the project’s aim was to develop a simplified 3D visual language that could simultaneously address the learning requirements of an audience of first-year medical students and expedite the production of quality animations in an often time and funding-strapped academic or other non-commercial context. It is also hoped that this paper can serve as a guide for first-time medical animators tasked with a similar project.

Project Background and Methodology

Challenges in medical education
The project was initially instigated by the Ninewells Hospital Department of Histopathology with the aim of incorporating more effective and efficient educational resources into the medical curriculum. Medical education is continuously expanding its scope to keep abreast of advances in the biomedical sciences and changes in clinical practice, such that instructors are placed in the challenging position of covering the contents of growing syllabi within limited teaching schedules. It is particularly critical to impart a solid knowledge base to first-year students, as the upper-year medical curriculum elaborates upon early training (University of Dundee, 2014). The challenge of teaching novices is compounded by the risk of overwhelming them with unfamiliar information; thus potentially reducing levels of interest, retention and engagement and jeopardising their learning. Classroom experience suggested that in the context of competing demands for their time and attention, the first-year undergraduate cohort often struggled to identify key learning points within pathology’s detailed coverage of disease pathways and mechanisms (see also Domizio, 2006).

Choice of topic
Considering the above points, it was decided to create a resource to tackle a ‘pathway-heavy’ topic, apoptosis. Apoptosis, also known as programmed cell death, is a physiological process by which old, diseased, or superfluous cells self-destruct, thus allowing new, young and healthy cells to replace them (Kerr et al., 1972). Abnormalities of excessive or insufficient apoptosis are involved in multiple pathologies, including cancer, autoimmune diseases and neuro-
degenerative diseases. The study of apoptosis is on the frontline of several branches of medical research, hence its inclusion in the first-year pathology curriculum.

Choice of medium and supporting research
A review of the existing literature pointed to animation as the medium best suited to this combination of topic (apoptosis) and target audience (first-year medical students). Multiple studies suggest that animation is particularly effective at promoting deep learning in novice learners, as compared to static visual, verbal, or multimedia resources alone (see the meta-analysis of 26 primary studies by Höffler and Leutner, 2007). Further research also implies that animations are even more effective at improving learners’ understanding and retention of topics in cell and molecular biology (Stith, 2004; McClean et al., 2005; O’Day, 2007).

It has been conjectured that animation is so appropriate for novice life science education because its structure is analogous to that of the content it communicates (McClean et al., 2005; O’Day, 2007). Much like the dynamic and transformative cellular events they describe, animations (particularly 3D animations) are visual, spatial, dynamic and sequential, such that beginner learners are spared much of the trouble of filling in the gaps as they map out a complex and often unfamiliar microscopic world (McClean et al., 2005). In cognitivist models of learning, such as cognitive load theory (Sweller, 1994) and the cognitive theory of multimedia learning (Mayer, 2005), this is described as facilitation of ‘essential processing’ and schema construction, the stages at which learners construct mental representations of the information they are taking in, as described by information processing theory (Mayer, 2005). The consequent reduction of cognitive load fosters more meaningful learning, including the integration of information into long-term memory and the ability to apply it creatively to new situations.

For all their potential benefits, animations are not fail-proof (Lowe, 2004); poorly made or non-adapted animations may even impede a beginner’s learning process by including distracting material (in Mayer’s terms, increasing ‘extraneous load’) or failing to encourage the learner to see the bigger picture (hindering ‘generative processing’). To help minimise this potential damage, Mayer’s nine prescriptive principles for the design of multimedia content were reviewed to be implemented where suitable (Mayer & Moreno, 2002, 2003). The first five principles aim to limit extraneous load. The coherence principle advises omission of irrelevant content and the redundancy principle similarly excludes empty repetition. Importantly, content should be highlighted as per the signaling principle, while the temporal and spatial principles suggest presenting corresponding words and images simultaneously and in close proximity. Two further principles aim to manage essential processing: content should be broken down into shorter chapters (segmenting principle) and important terms explained before they appear in context (pre-training principle). Finally, to foster generative processing, Mayer proposes a ‘voice principle’ and an open-ended ‘personalisation principle’, as learners were found to respond to voice-over narration in animations better than to text and to prefer a conversational tone to more formal speech.

Whilst these principles value simplification, further research suggests a more nuanced view of cognitive load and representational reductionism. Indeed, complex, more realistic representations of molecular-level processes may in fact help students build fuller mental schemas and prepare them for more advanced learning stages (Jenkinson & McGill, 2012, 2013). One of the main challenges of designing educational resources for beginners is thus to control the complexity of information—to simplify without abstracting to the point of denaturing the content.

Going bespoke
In a non-commercial, academic context, the initial strategy is often to seek public domain or creative commons resources for reuse or remixing. However, a search through the pool of existing animations revealed no resources appropriate for a neophyte medical student audience.Most were either too representationally complex or insufficiently explained. It was decided that a bespoke animation would be produced. Whereas bespoke molecular animations are often costly, research- and production-heavy works, this production was unfunded and faced a limited workforce and time frame, so the challenge further concretised into developing a visual language that would satisfy both educational and production requirements.

Design Process

Overview
The project was broken down into three main phases to follow a standard animation workflow: pre-production, production, and post-production, with each phase involving four stages. All work was performed on a late 2013 15’ Retina Macbook Pro running OS X 10.9.5 with a 2.3 GHz Intel Core i7 Processor and 16 GB DDR3 RAM at 1600 MHz DDR3.
**Pre-production**

To begin the pre-production phase, a project brief was drafted to finalise the nature and scope of the commissioned work, the learning objectives to be met, the target audience, the general art direction and the final deliverables. It was agreed that the medical artist would produce a short entry-level animation focused on key topics. Brevity would help keep the audience’s attention, but just as importantly it would enable the medical artist to meet the three-month production deadline. Voice-over narration would clearly expound the content (personalisation and voice principles). Labels would help highlight important or technical terms (signalling principle, see also Jantzen et al, 2015). A musical score would carry the film’s flow and complement the narration.

As per the coherence principle, learning objectives were carefully limited to five key points focusing on the initial stages of apoptosis (morphological changes of the dying cell would be described in a future animation). It would be explained that:

1. There are extrinsic and intrinsic pathways of apoptosis.
2. The common endpoint for both pathways is initiation of the caspase cascade.
3. The extrinsic pathway relies on the binding of Fas to FasLigand and stepwise progression to the Fas Associated Death Domain (FADD).
4. The intrinsic pathway involves pro-apoptotic factors Bax and Bak.
5. The intrinsic pathway promotes ‘leaky’ mitochondria with release of molecules that initiate the caspase cascade.

Taking a further cue from cognitive load theory, it was decided that the animation would be produced in 3D to better convey the specific geography and spatial character of apoptosis. 3D animation software simulates perspectival space and offers powerful lighting and material simulations tools to enhance the sense of the image’s depth and realism (without however necessarily committing the production to photorealism). Viewers would thus expend less time and effort translating two-dimensional information into a three-dimensional mental schema. The final animation output was established as an HD video coded in a H.264 MPEG-4 AVC format at 25 frames per second. At this frame rate, render loads would stay manageable on the available hardware; the resultant video would also be suitable for either broadcast or digital distribution.

A script covering all the learning objectives was developed through a series of edits that refined the narrative arc and the narration’s phonetics and conversational rhythm, pacing the script at 120 words per minute for a final count of 455 words.

**Figure 1** A sample storyboard slide.

**Action:** Callout to intracellular caspase breaking up a string of proteins as the cell continues breaking down in the background.

**VO:** In apoptosis, the cell is broken down from within by proteins called “caspases”.

**Text Displayed:**

Caspase
seconds and frames was worked out with an online frame calculator (zapstudio, 2016). This information was essential to setting up scenes in Maya, where the action and camera movements must be timed in relation to total scene duration.

As a list of shots and models was being prepared ahead of the production phase, the art direction was further concretised. Indeed, the initial representational choice of generic ‘blobby’ proteins at the storyboard stage was by then deemed misleading. The molecular representation of proteins imitated in these sketches is based on strict graphic conventions devised to convey detailed information about protein structure; to mimic it arbitrarily without undertaking the lengthy research into the topology and conformational changes of over a dozen proteins could arouse confusion or suspicion.

Since the proteins’ molecular makeup was not

Figure 2 The Adobe Premiere CC workspace during the animatic phase, showing the storyboard stills edited to the voice over.

Figure 3 Concept sketches of apoptotic pathway proteins.
part of the learning objectives, a ‘3D diagram’ approach was devised in its stead. Elements of the virtual cell environment would be illustrated in three dimensions, but as simplified yet geometrically expressive characters, as in a diagram (Figure 3). In this way, different protein families and their interactions could be emphasized, often through analogous shapes, such as interlocking female and male parts. For instance, to describe the intrinsic apoptotic pathway, pro-apoptotic proteins were illustrated as screw shapes that bind to each other along their threads to bore channels into the mitochondrial membrane, thus releasing apoptosis-triggering cytochrome-c. Similarly, anti-apoptotic proteins were illustrated as springs that can fasten onto the ‘screws’ to prevent their binding action. These same spring shapes can in turn be blocked by protein ‘caps’ that prevent the interlocking of pro- and anti-apoptotic proteins. This simplified approach avoids the ‘extraneous load’ that would be encountered through a more scientifically accurate representation but still helps the learner to grasp the learning objectives and generic concept.

It was also conjectured that the characters’ slightly anthropomorphic shapes and behaviours would help individuate them and make them more memorable. Additionally, students could easily sketch the simple shapes in their own notes, a potentially handy mnemonic technique.

A simplified geometry offered the additional benefit of expediting production by streamlining the modelling and animating processes. The time saved would instead be invested in other phases of production and post-production, for instance to improve motion and lighting effects.

Production
Production unfolded mostly in the 3D workspace (Autodesk Maya 2015) and was divided into four stages in which each scene, set up according to its previously calculated duration, was modelled, animated, lit and rendered (Figure 4). Production also required some 2D work, such as the creation of backgrounds in Photoshop. Although production is an iterative phase focusing on the refinement of successive cuts, it will be described linearly for brevity.

Production I: Modelling
For the modelling stage, a variety of polygonal modelling techniques available within Maya 2015 were employed. Polygon geometry was generally preferred to NURBS and subdivision surfaces because it accepts a wider range of materials (shaders).

In the most direct technique, polygon primitives (shapes such as cubes or spheres) were modified to form more complex objects. Other simple symmetrical shapes were generated by revolving a curve around an axis to create a NURBS volume that was then converted to a quad-based polygon.
More complex geometries were derived using nParticles, a feature of Maya’s dynamic simulation framework. The apoptosing and cancer cells were both modelled and animated through ‘metaballs’, which allows elements to ‘blob’ together or conversely to separate viscously (Figure 5).

Other modelling techniques included the use of displacement and bump maps (for close-ups of the cell membrane, for instance) and of custom geometry-generating scripts (for the section through the mitochondrion’s phospholipid bilayer).

Production II: Animating
The 3D models were animated in several ways, the most basic being keyframing. In keyframing, the start and end states are specified so that Maya can interpolate the intermediate states over the given time span. For instance, the spatial coordinates of objects can be keyframed to move them across the virtual space. In the cases of protein interactions in which multiple objects were involved, such as various bindings or caspase activations, it was easiest to begin with the desired end state and work backwards on the timeline from there.

Nearly all Maya elements can be keyframed, including cameras, simulations, lights and shaders. The cameras were set up to match the framing of each shot as shown in the storyboard and camera movements were keyframed to track, zoom, pan, or dolly. Certain material attributes (shaders) were keyframed as well, notably in the case of the foetal hand, to highlight the death of the cells in the web spaces. The foetal hand itself was animated using a blend shape deformer, which defines a target shape that an initial shape morphs into via keyframing. The proto-hand’s fingers in their webbed state thus transition to a hand with fully defined fingers.

Parts of the animation were scripted with expressions, such as the rotational value of the caspase’s ‘sawblade’, which is based on the current frame number on the timeline to simulate a continuous rotation. That expression was keyframed to be enabled only upon caspase activation.

Finally, Maya’s nParticles emitter system was ideally suited to animating the cytochrome-c leakage from the mitochondrion (Figure 6) and the disintegration of the actin filaments in the final steps of the intrinsic pathway.

Production III: Lighting and Shading
The lighting scheme was kept simple, with enough soft shadows on the lit volumes to indicate depth whilst avoiding harsh and distracting dark shadow lines. Although additional light sources were occasionally used for emphasis, most scenes were lit by a Physical Sun and Sky (PSS) environment, which simulates outdoor lighting conditions with a directional light source. PSS shadows were softened directly through an attribute in the physical sun node, but also by changing the haze settings in the physical sky. Solar rays were also angled to simulate a different quality of light based on time of day. To maintain continuity between one scene and the next when travelling in and out of cells, the lighting was set up to consistently emulate conventional scientific illustration lighting, namely, coming in from the left-hand side at a 45 degree angle with respect to the image plane.

Maya’s library of materials (known as shaders) works with its lighting system to simulate textures and other effects. Three main shader types were used: lambert, mia_material_x and ramp shaders. The lambert, being the simplest and fastest-rendering of the three, was used where only colour was required,
for instance for the internal mitochondrial membrane or the pro-apoptotic proteins. Mia_material_x renders transparency better than the lambert, and was applied to the outer mitochondrial membrane. The apoptosing cell was assigned a more complex ramp shader, with varying levels of incandescence, transparency and colour applied from its centre to its edge (Figure 7). In this way the cell nucleus could be represented without additional modelling and smoothly followed the movements of the membrane during cell breakdown or replication.

Cross-animation style frames were created to ensure chromatic consistency throughout the project (Figure 8). Chromatic cues were also used to indicate a scene’s location: blue backgrounds for multicellular environments, green ones for intracellular shots. Other cues also helped generate certain associations and highlight important features: for instance, the caspases’ bright, red tones were meant to communicate a state of emergency within the cell, whereas the use of duller colours for cancer cells better suggested disease.

Production IV: Rendering
During rendering, a render engine calculates what a camera ‘sees’ across a sequence in a given scene file and generates hundreds of corresponding still images, converting each second of footage into 25 separate frames. For this project, Maya’s mental ray render engine was used with HD production settings, yielding an output of 1920 x 1080 pixel tiff-format images with alpha. The alpha setting removes any background from the render so that the images of the rendered models can be brought into After Effects with the transparency preserved. Render settings were kept basic: playblasts (non-rendered, video-output animation previews) were used to edit the two rough cuts, and no render layers or passes were used for the fine cuts. However, final gathering and ambient occlusion (two algorithms for realistically rendering the way light bounces off surfaces) were applied at render time to improve the image quality.

Production
Post-production I: Compositing
In order to convert the renders into a video-friendly format, the stills sequences were first composited in Adobe After Effects and layered above static backgrounds created in Adobe Photoshop CC 2015.

Figure 7 A ramp shader applied to a single piece of geometry was used to simulate both the cell membrane and nucleus.

Figure 8 Styleframes for “What is Apoptosis?”.
Compositing was speedy as the scenes were rendered to be used ‘out of the box’ with little additional processing. Multiple compositions were then imported directly into video editing software Adobe Premiere to be edited with the voice-over and score, before being exported to a suitable video format. More complex transitions such as crash zooms were assembled directly in the After Effects timeline, but straight cuts or fades were left for the final edit in the video editing software Adobe Premiere CC 2015.

Post-production II: Motion graphics – titles and labels
Simple motion graphics were added in After Effects, directly within the various scene compositions. Since text is displayed on-screen for no more than a few seconds, viewers should be able to read it quickly and effortlessly. A neat and elegant sans-serif typeface, Mr Eaves Modern, was selected from the Adobe Typekit to promote legibility and fit the overall animation aesthetic: it features simple and direct letterforms with crisp edges that do not blur during rendering. Uppercase lettering was used for titles and italics were used for captions and labels.

Post-production III: Audio tracks
Audio tracks were added into Premiere. Whilst the scratch voice-over track used in the early cuts was recorded with a laptop microphone onto Quicktime, a more professional device (a Snowball USB condenser microphone by Blue Microphones) was used for the final track, which was recorded directly into Adobe Audition CC 2015. A homemade ‘pop’ filter was used to minimise plosives in the recording. If a mistake was made during the narration, the recording was kept running to allow for a corrected version of the sentence to be enounced, so that the mistake could be edited out subsequently. The track was then sound corrected to remove background and breathing noises and to improve the clarity of the voice-over.

The score, a track entitled ‘Enrichment’ from Podington Bear’s Backbeat album, was downloaded under a creative commons license from the free music archive. It was chosen for its calm, non-distracting quality.

Various sound effects were downloaded from websites such as freesound.org and freesfx.co.uk: pops, clicks, whooshes and bubbling sounds were added to emphasise on-screen events. Playing with the right and left balance of each sound helped create movement and depth within the sonic landscape. The sound effects also balanced the pauses in the narration, allowing for a slow enough pace for the material to sink in without becoming boring.

Post-production IV: Export and upload
The final cut was exported from Adobe Premiere with H.264 1080p settings, ready for upload onto the TILT Vimeo account (https://vimeo.com/178595352) and YouTube (https://youtu.be/-vmtK-bAC5E). The online animation can be viewed from a computer, tablet or smartphone.

Feedback
Throughout the project and upon its completion, internal feedback circulated between the pathology and medical art departments as well as TILT.

The animation was then screened to an audience of first-year University of Dundee medical students during a pathology lecture on October 14, 2016. The students were asked to complete an anonymous online survey that had been approved by the University Centre for Anatomy and Human Identification’s ethics committee prior to distribution. In total, 31 students participated (22 female respondents, nine male). As shown in Figure 9, eighty-seven percent (87%) of respondents stated the animation had increased their general understanding of apoptosis. Over 90% reported that it had improved their grasp of the intrinsic and extrinsic pathways and the caspase cascade, with one student commenting ‘I felt the animation made the different pathways a lot clearer in my head’.

The animation also seemed to increase their interest in the study of pathology: whereas 30% reported being very interested in the subject before watching the animation, that number increased to 53% after viewing (Figure 10).

| How has the animation contributed to your understanding of the following topics? |
|---------------------------------|-----------------|-----------------|
| DECREASED | UNCHANGED | INCREASED |
| Apoptosis  | 13% | 87% |
| The extrinsic pathway | 10% | 90% |
| The intrinsic pathway | 10% | 90% |
| The caspase cascade | 10% | 90% |
| The physiological relevance of apoptosis | 29% | 71% |
| The pathological relevance of apoptosis | 29% | 71% |

Figure 9 Evaluation of the animation’s effectiveness in communicating key learning objectives.
With respect to the art direction, most students found the animation to be well-paced, of appropriate length and easy to follow (Figure 11). 93% of students agreed or strongly agreed that the diagrammatic protein representation was fitting, 7% stayed neutral. One student mentioned that ‘it helped differentiate the proteins and understand their functions’ and another found that ‘the shapes and colours made it easy to follow and gave me an image to work with in my own head when learning about the topic further’.

Yet another wrote that they ‘thought the animation was very clean and focused as there wasn’t a lot [...] happening on the screen at once, so that helped to convey the point much more clearly.’ A minority, however, would have appreciated some additional detail, but not so much as to ‘overload and become disengaging’.

Respondents were more divided regarding the soundscape. Whilst most found the script useful and the narration clear, others commented on personal preferences regarding the use or non-use of music.

All students agreed that the animation was professional and well produced, with one student’s comment highlighting the instructional strength of high production values: ‘very professional animation, which makes it easier to follow due to good quality animation, script and sound effects.’

In terms of how animations fit into their study habits, most students thought that they would be most likely to use animations of this type as revision aids to help them focus on key information. Some used the animation to take notes, pausing the video when needed. Several commented that they would also find such animations useful as introductions to more detailed lectures. ‘I just loved it, I wish there were more videos that explain other topics also.’

Respondents would also like to see similar resources for subjects that describe ‘complicated processes that students (especially undergraduates) may not understand without it being visualised’, with immunology frequently mentioned in their requests.

Most students watched the animation twice: first in class, then before filling out the survey. Many stated they were already familiar with educational animations (Figure 12). Of those who were not regular animation-viewers, a few commented that they would start seeking out such videos more often on the basis of their positive experience with this particular resource.

As for possible improvements, one student would have liked to see a more detailed depiction of how the apoptosome activates the caspase cascade. Another suggested a more explicit summary of important points at the end to consolidate the information.
Discussion

Overall, the animation was well received by professionals and students. Student comments in particular provided encouraging initial validation of both the art direction and of the application of the multimedia design principles. Indeed, the visual simplification and segmentation appear to have contributed to the production of an effective and targeted medical animation consistent with cognitive learning theory models. Many comments highlight the importance of such a ‘minimalist approach’ aimed at reducing extraneous load when learners encounter complex concepts and mechanisms for the first time. The comments also, however, highlight that establishing the balance between reductionism and oversimplification can be difficult. As with any design-based process, an approach empathic to the user should be adopted and future animations may avoid this issue by more engagement with the user audience throughout the early design process.

Additionally, since time constraints limited collection of student feedback to an online survey, actual, rather than perceived, knowledge retention and integration were not assessed. A more formal effectiveness study could be carried out amongst a future first-year cohort, in which students could be tested on their understanding of apoptotic processes after either watching this animation or consulting more conventional teaching resources. A further comparison could involve one group watching this simplified animation, and another being taught with a more complex animation of similar duration, for example Apoptosis by Drew Berry (Berry, 2007). Such comparative studies could help test the limits of representational simplification and determine with greater verifiability, for instance, whether the animation was pitched at the appropriate level and what features of the animation were most instructionally effective.

The simplified 3D language also enabled a timely completion of the project, such that the project’s goal of developing an art direction that could allow for speedy production of this and subsequent resources was also met. This language could be easily reproduced in future animations, without the need for extensive knowledge of molecular biology.

For an even speedier turnaround time in future projects, more thought could be given to how to draw on pre-existing creative commons resources and minimise time spent in Maya. Moreover, although the animation included closed captioning, accessibility could be further improved, for instance by optimising the colours of on-screen text for colourblind viewers.

Beyond the immediate classroom impact of teaching aids such as animations, it is interesting to consider how such assets fit into the greater picture of medical education. Posting the animation online, for instance, ensures that all Dundee students and the wider public alike have access to this resource, and as of July 2018, the animation had garnered over 122,000 views, thus reaching even more students and resulting in greater exposure for the University. Additionally, the animation was licensed under a Creative Commons CC-BY-NC-ND 4.0 licence, to be freely copied and redistributed for non-commercial use (with credits given). Sharing of such teaching resources contributes to the maximisation of assets and to the development of FOAMed (Free Open Access Medical Education), in addition to further increasing a department’s reach, profile and visibility. It similarly offers medical artists the opportunity to highlight their profession’s contributions to academia.

Conclusion

In order to keep medical education apace with advances in biomedical research and practice, many instructors address new teaching challenges by diversifying their repertoire. Some resort to e-learning resources, yet the offer is not always consistent nor reliable: although a wide range of media has thus been introduced into medical education, the sheer volume of available content can further distract and overload learners, particularly if the resource is poorly produced or ill-adapted to its audience.

This case study for a new and bespoke teaching resource for the pathology curriculum began by identifying a general need, namely, how to improve first-year medical students’ grasp of cell signaling, then targeted a particular instance of this topic, the apoptotic pathways. After researching the most suitable formats for both subject and audience, the team produced a short and uncluttered narrated 3D animation, which was then shown to students and shared with the general public. Favourable feedback suggest that there is indeed a demand for such animations and that the chosen design approach could yield further positive results. Moreover the workflow was explicitly designed to streamline the production of molecular biology animations, in the hopes that more such resources could subsequently be created and publicly shared in the future.

Disclosure statement

The authors report no conflicts of interest.
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