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RESEARCH NOTE

Low-cost, rapidly-developed, 3D printed *in vitro* corpus callosum model for mucopolysaccharidosis type I [version 1; peer review: 2 approved]

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Abstract

The rising prevalence of high throughput screening and the general inability of (1) two dimensional (2D) cell culture and (2) *in vitro* release studies to predict *in vivo* neurobiological and pharmacokinetic responses in humans has led to greater interest in more realistic three dimensional (3D) benchtop platforms. Advantages of 3D human cell culture over its 2D analogue, or even animal models, include taking the effects of microgeometry and long-range topological features into consideration. In the era of personalized medicine, it has become increasingly valuable to screen candidate molecules and synergistic therapeutics at a patient-specific level, in particular for diseases that manifest in highly variable ways. The lack of established standards and the relatively arbitrary choice of probing conditions has limited *in vitro* drug release to a largely qualitative assessment as opposed to a predictive, quantitative measure of pharmacokinetics and pharmacodynamics in tissue. Here we report the methods used in the rapid, low-cost development of a 3D model of a mucopolysaccharidosis type I patient's corpus callosum, which may be used for cell culture and drug release. The CAD model is developed from *in vivo* brain MRI tracing of the corpus callosum using open-source software, printed with poly (lactic-acid) on a Makerbot Replicator 5X, UV-sterilized, and coated with poly (lysine) for cellular adhesion. Adaptations of material and 3D printer for expanded applications are also discussed.

Keywords

3D printing, neurodegenerative disease, cell culture, *in vitro* release, mucopolysaccharidosis, corpus callosum

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Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

Mucopolysaccharidosis (MPS) is a spectrum of inheritable conditions involving the accumulation of glycosaminoglycans (GAGs) following disruption of key lysosomal enzymes, which in turn leads to complications on a cellular, tissue, and organ level¹. In MPS type I (MPS I), which is characterized by a deficiency of the enzyme α -L-iduronidase, brain MRI scans reveal thinning of white matter and lesions within the periventricular area and especially the corpus callosum (CC)². The CC is the largest white matter structure in the brain, with more than 300 million axonal projections, and it interconnects the left and right hemispheres³. MPS I leads to patient-specific, irregular white matter density and geometry in the CC. Current treatment for MPS include enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT). ERT has been shown to ameliorate MPS symptoms, yet does not prevent disease progression⁴, owing in part to poor bioavailability. HSCT has been shown to improve cognitive development. Donors, however, can be hard to find unless umbilical cord blood is available; the procedure also has significant health risks^{5,6}. As such, more research into potential targets and drug delivery excipients, which can provide tunable release kinetics, is needed to develop a library of promising treatment options. Additionally, owing to the highly patient-specific deterioration of cerebral white matter, patient-specific identification of synergistic drug combinations and optimal drug release kinetics can enable a more personalized medicine approach to treat MPS in the future.

Traditional methods of screening use two-dimensional (2D) cell culture to study biochemical pathways and targets in cells. Yet, 2D designs of traditional cell cultures fail to account for complex cell-cell and/or cell-matrix interactions. There has been a growing literature that demonstrate the importance of three-dimensional (3D) environments in expressing phenotypes, genes, and proteins at levels found *in vivo* and not otherwise seen in 2D models⁷⁻⁹. 2D *in vitro* drug release studies of promising therapeutic targets are generally limited to providing qualitative insight into *in vivo* release behavior. Seemingly arbitrary choices in probing conditions such as material volume, material surface area, supernatant volume, and rotator conditions, hinders quantitatively rigorous conclusions of mass transfer, pharmacokinetic, and pharmacodynamics properties to be made from benchtop measurements. These in tandem demonstrate a pressing need for the use of 3D disease models as a more representative *in vitro* system. Here, we describe an inexpensive and fast method of developing such patient specific 3D models.

Methods

The 3D brain MRI scans of a 20-year-old male subject with MPS I and an age-matched healthy male control were manually traced to obtain a 3D structure of the corpus callosum (CC). The 3D model was printed on a Makerbot Replicator 5X, sterilized (Figure 1), and could be used for cell culture or *in vitro* release studies. The de-identified MRI scans were obtained as Digital Imaging and Communications in Medicine (DICOM) files (Dataset 1¹¹). The CC was traced on the mid-sagittal slice and five adjacent slices in each hemisphere using open source InVesalius 3 (<http://www.cti.gov.br/invesalius/>, RRID: SCR_014693). Alternatively, OsiriX 8.0.1 software (<http://www.osirix-viewer.com/>, RRID: SCR_013618)

may also be used. The software was then used to render the scans into a single .STL file (Dataset 2¹²). The 3D model of the CC was loaded into MakerBot Desktop v. 3.6.0.78 (<https://www.makerbot.com/download-desktop/>) and printed on a MakerBot Replicator 5X with poly(lactic acid) at a resolution of 0.2 mm, maintaining life-size dimensions. Stratasys post-processing fluid was optionally used to remove any support material. The 3D printed structures were rinsed with a 70% ethanol/water solution and UV-sterilized overnight. The prints were then coated with polylysine (Sigma) for cellular adhesion, by dipping them upside down in a 0.5 mg/mL poly-L-lysine solution for at least 10 minutes. Only the top of the surface was dipped (Figure 1d), as this was the area of interest where the drug delivery materials would be loaded, but for other applications discussed in the next section, the entire structure can be dipped into ~50 mL of the poly-L-lysine solution for complete cell adhesion on the top and bottom.

For cell culture, this object would be used in a sterile flask, or alternatively, a larger sterilized container. For use as a drug delivery platform, the object could be both be cultured with cells as previously discussed and kept in cell culture media, or used without cells in PBS. A preteral drug delivery system, such as shear-thinning hydrogels or hydrophobic polymer melts, can be injected with a syringe on top of the 3D model, until the amount of drug loaded is comparable to translational doses, or until the material completely coats the 3D model. When a cell coated surface is used, the drug will be released into the cell media. Conversely, when a cell-free model is used, the drug release kinetics are monitored in PBS, which is less prone to interference. Care must be taken to ensure that the release kinetic probing molecule's absorbance or emission spectra are not greatly interfered with by cell culture media.

Figure 1. 3D models and prints. (a–b) T1-weighted brain MRI with resolution of 1×1×1 mm, midsagittal slice, arrow pointing at corpus callosum in (a) healthy control and (b) MPS I subject. (c) CAD image of MPS I corpus callosum taken at five adjacent slices in each hemisphere. (d) 3D printed MPS I corpus callosum with poly(lactic-acid) on a Makerbot Replicator 5X. (e) UV sterilizing the print overnight.

Dataset 1. DICOM files for the de-identified MRI scans of the corpus callosum of a MPS I subject

[10.5256/f1000research.9861.d144327](https://doi.org/10.5256/f1000research.9861.d144327)

Dataset 2. Resulting CAD file from InVesalius 3 software (.STL), used to render the DICOM files in Dataset 1

[10.5256/f1000research.9861.d144328](https://doi.org/10.5256/f1000research.9861.d144328)

Discussion

This technique uses, but is not limited to, poly(lactic-acid) (PLA), a readily available filament for desktop 3D printers, such as the Makerbot Replicator 5X. PLA has been widely shown to be biocompatible¹³. Applications of this platform include studying *in vitro* drug release of injectable drug depots for delivery of therapeutics e.g. proteins for enzymatic deficiency disorders, such as MPS, and hydrophobic small molecules for brain cancer. *In vitro* drug release methodology can largely vary release profiles depending on the geometry of the container used. A 15 mL conical tube provides a different area for mass transfer than a 10 cm culture dish or 5 mL glass vial. This 3D modeling platform can potentially offer a more realistic and more standard geometry for monitoring drug release. Additionally, many therapeutic approaches to treat brain cancer and other diseases rely on injecting or implanting material that maintains a high interfacial concentration to improve drug bioavailability and efficacy, such as gold nanoparticle radiosensitizers for radiotherapy^{14,15}. The drug depot material can be tested *in vitro* on this platform to determine the proper interfacial concentration given to-scale surface area of the tissue, and monitor the duration to which this concentration can be maintained.

Figure 1 (a–b) demonstrates the thinning of the CC in MPS I (b) compared to that of a healthy brain (a). Given the uniqueness of each MPS patient's brain pathology, density, and geometry, the ability to test the therapeutic window, effectiveness, and optimal drug loading concentration into an injectable drug depot for each specific patient is highly useful. A team of high school and undergraduate students was able to render the CAD file (Figure 1c) and 3D print on common desktop 3D printers at a low cost (Figure 1d), suggesting that this culture may be scaled more readily than expensive 3D *in vitro* platforms. This material's modulus is approximately 3 GPa, several orders of magnitude larger than native tissue. In order to

create a 3D cell culture platform which enables cell migration and proliferation within the tissue, a 3D bioprinting approach must be used^{16,17}. In conclusion, this method's robustness, ease, and low cost make it adaptable for use for a wide variety of applications in drug delivery, drug discovery, tissue engineering, and stem cell biology.

Data availability

Dataset 1: DICOM files for the de-identified MRI scans of the corpus callosum of a MPS I subject, doi: [10.5256/f1000research.9861.d144327](https://doi.org/10.5256/f1000research.9861.d144327)¹¹

Dataset 2: Resulting CAD file from InVesalius 3 software (.STL), used to render the DICOM files in Dataset 1, doi: [10.5256/f1000research.9861.d144328](https://doi.org/10.5256/f1000research.9861.d144328)¹²

Ethics

The study protocol involving the brain MRI acquisition was approved by the University of Minnesota IRB committee. Written, informed consent to publish results from MPS patients and healthy volunteers was obtained.

Author contributions

AT, SS, and IN developed the experimental outline. AT, MG, SS, SC, AM, DG, BH, and RT developed the model. AT, SS, AM, DG, and IN wrote the first draft of the paper. All authors revised the manuscript and have agreed to the final content.

Competing interests

No competing interests were disclosed.

Grant information

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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The authors motivate the problem well by summarizing the drawbacks of 2D cell culture in vitro studies to predict in vivo responses in humans.

3D human cell cultures offers several advantages; here, the authors highlight how, in this era of personalized medicine, 3D platforms can be tailor-made to offer a more realistic approximation of in vivo studies when examining drug release kinetics. Specifically, a 3D printed model of a mucopolysaccharidosis type I patient's corpus callosum is developed from an MRI trace.

The report is presented clearly and the authors demonstrate how to rapidly develop the aforementioned 3D model and prepare it for drug delivery studies. However, the paper lacks any studies that demonstrably show the advantage of the 3D model. Future work that experimentally establish the effectiveness of 3D models synthesized using the reported method would be useful.

Competing Interests: No competing interests were disclosed.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 19 January 2017

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Dustin Sprouse

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Indeed our society and health care system is moving toward more personalized medicine and care. Currently this is expensive and there is a great need for high throughput screening, lower costs, and 3D human cell culture to verify new drug discovery.

The authors sum this up well in the title and introduction and the brief follows this idea throughout and provides an example with the rare genetic disorder of mucopolysaccharidosis.

The paper flows nicely, but it was the methods section that had a lack of details and data and leaves one with more questions than revelations. The data is available in two linked files; however, one would first need the correct software to open them. Thus, I recommend more images/video to convey what the scientists accomplished and show the readers the potential results and outcomes.

The discussion sums up some important ideas that need to be considered when developing a methodology such as this one. These methods will need to be independently verified and show promising potential prior to a new mass platform.

In conclusion, this paper puts forth the ideas of a low cost approach to 3D printing scaffolds for drug release profiles, hopefully leading to faster turnarounds and lower costs of personalized medicine, drug discovery, tissue engineering, and stem cell biology.

Competing Interests: No competing interests were disclosed.

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