High-Throughput Processing to Preserve Viable Cells: A Precision Medicine Initiative Cohort Program Workshop

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Conventionally, biobanks supporting clinical research studies have preserved serum, plasma, urine, saliva, a variety of tissue types, and stool. With the emergence of increasingly sophisticated technologies for analyzing single cells, there is growing interest in preserving viable blood cells for future functional studies. The new *All of Us* Research Program (formerly the Precision Medicine Initiative Cohort Program) biobank plans to house samples from a million or more individuals as part of a cohort with rich phenotypic data and longitudinal follow-up (www.nih.gov/research-training/allofus-research-program). Storage of viable cells for future single-cell analysis offers the promise of new biology, discovery of novel biomarkers, and advances toward the goal of precision medicine. A workshop was held in the summer of 2016 to evaluate the case for preservation of viable mononuclear blood cells and its feasibility within the collection plan for the biobank.

Keywords: single-cell analysis, high-throughput, cell viability, cell preservation, precision medicine, biomarkers

Introduction

COLLECTING AND PRESERVING samples from clinical studies add to the value of those studies by allowing additional scientists to test new hypotheses without the need to collect the samples or associated data. Most biobanks in the United States store primarily whole blood, plasma, and solid tissues, under conditions that do not preserve viable cells suitable for functional analyses. While many surveyed biobanks store immortalized cell lines that contain viable cells,¹ the cells are transformed, resulting in distinct differences from the original primary cell.

There are many advantages to working with viable cells, which allow researchers to carry out functional studies in which cells respond to stimuli, medications, or specific conditions, as opposed to the static, observational studies that are carried out with preserved cells and tissues. Thus, for example, it is possible to study the transcriptional response of viable cells over time when exposed to a specific medication rather than the current approach of looking at the messenger RNAs (mRNAs) present in the preserved cells of an individual taking the medication. With viable cells, it is possible to measure immediate and subsequent transcriptional responses over time, whereas the readout from stored cells is a single snapshot of cellular responses that may reflect both the initial response to the medication and subsequent physiological adjustments. The more detailed molecular observations permit more sophisticated an-

alyses to relate transcriptional response to the participant's medical history of response to the medication. Or, in another example, using stimulated immune cells may reveal early inflammatory markers that reflect a response to cancer or autoimmune diseases, and that would not be detectable in preserved cells.

Peripheral blood mononuclear cells (PBMCs), if cryopreserved properly, can retain their viability. However, there is not an off-the-shelf, automated, or high-throughput process or instrument to perform the requisite isolation and cryopreservation, so this has not been a common feature in large biobank collections or collections associated with large, well-characterized cohorts. Cryopreservation of PBMCs is currently a manual and expensive process.² As will be detailed in subsequent paragraphs, reagents, specialized cell separation centrifuge tubes, and additional labor or automation are required to produce these additional biospecimens. Cost is weighed against the additional value the biospecimens provide, based on their quality, the cohort they represent, and available assays that depend on viable cells.

The *All of Us* Research Program, formerly known as the Precision Medicine Initiative Cohort Program, is a National Institutes of Health (NIH)-funded project designed to recruit a million or more individuals across the United States for longitudinal follow-up. The working group charged with developing the blueprint for this U.S. Presidential initiative identified several high-value scientific opportunities that would be enabled by

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this program, ranging from “identification of determinants of individual variation in efficacy and safety of commonly used therapeutics” to “discovery of biomarkers that identify people with increased or decreased risk of developing common diseases.”³ These and other compelling research study opportunities will take advantage of a large, well-characterized cohort, electronic health records, data on environmental exposures and genetic factors, mobile health technologies, and biospecimens. The newly created *All of Us* biobank will store samples from each participant. This biobank will operate with a high level of automation due to the very large number of samples anticipated. The establishment of this major resource offered an opportunity to consider the value and feasibility of preparing and storing a sample of viable cells from each participant for future analyses. In August 2016, NIH sponsored the “High-Throughput Processing to Preserve Viable Cells: A Precision Medicine Initiative Cohort Program Workshop” in Bethesda, Maryland. In the first session, researchers using single-cell analysis made the case for the value of preserving viable cells. In subsequent sessions, repository scientists presented data about processes to preserve cell viability and solutions for automation of the process. The agenda and a list of the 25 attendees can be found at www.nih.gov/allofus-research-program/events.

Use Cases for Viable Cells

James Eberwine (University of Pennsylvania), Paul Robson (The Jackson Laboratory), John Tsang (National Institute of Allergies and Infectious Diseases), and Holden Maecker (Stanford University) each described their cutting-edge research using single cells or small numbers of cells.^{4–7} Live cells allow the study of chromatin dynamics, single-cell mRNA translation, enzymology, and functional genomics, all of which are not possible with fixed cells. Enabling technologies, including high-throughput single-cell transcriptomics and mass cytometry, are expanding use cases for single-cell analysis and are becoming increasingly accessible to researchers.

In the subsequent discussion, the attendees compared the number of cells required to carry out the analyses that they had described, as related to the number of cells in typically preserved blood volumes. This ranged from 20 to 50 cells for a transcriptomic signature⁸ to 1 million cells for mass cytometry measurements with a panel of antibodies.⁷ Other meeting participants suggested that 1000 cells would be a typical input for detecting low-level mRNA transcripts. The clear conclusion from the discussion was that the cell number required to perform the analysis varies by the assay being used and the question being asked. As a result of technological advancements, the sensitivity of assays for measuring proteins or transcripts or other analytes in cells is likely to improve over time, allowing for a smaller starting biological material input than would be required if the assay was performed today.

The group then turned its attention to preservation of viable cells. Allison Hubel (University of Minnesota) and David McKenna, Jr. (University of Minnesota) identified factors in the preanalytic phase that influence viability, including temperature, cell concentration, storage solution and osmotic concentration, and the need to standardize the postthaw timing until the assay is performed.^{2,9} Several presenters noted that the recommendations from the Biomedical Excellence for Safer Transfusion collaborative on best practices for collecting, transporting, and preserving blood stem cells^{10,11} may prove useful for developing the *All of Us* protocol. There was a

consensus, based on the literature and on the data presented that the cells in the blood are stable for up to 24 hours after collection for most subsequent analytic purposes. Helen Moore (National Cancer Institute, NCI) summarized NCI’s work to collect evidence-based practices for biospecimens and assess the extent of their use in the biobanking community (more information is available at <https://biospecimens.cancer.gov>).

The key question is whether it is possible to standardize and automate the isolation of viable cells in the setting of a biobank serving a very large cohort study. Rohit Gupta (Stanford University) described unpublished data on standardization of methods for isolating PBMCs for the Accelerating Medicines Partnership, comparing various cell separation tube technologies, preservation media, and freezing devices. Typically, density gradient centrifugation methods are used, and a number of tube systems (containing anticoagulant, density medium, and a gel or disc barrier) are available to facilitate PBMC separation. CPTs (Cell Preparation Tubes; BD) can be processed in an automated fashion, and a new system, SepMate (StemCell Technologies), also has the potential for automated processing. While these tubes are compatible with liquid handling systems, there are technical challenges when blood volume is low or hematocrit is high, and customized image analysis algorithms may be necessary to permit detection of relevant cell and fluid layers within the tube after centrifugation. Other tube systems such as Streck Cyto-Chex BCT and the Smart Tube System offer advantages for downstream flow or mass cytometry, but do not preserve cell viability or contribute to isolation of PBMCs.

Heiko Zimmermann (Fraunhofer-Institut für Biomedizinische Technik) and Wim Ammerlaan (Integrated Biobank of Luxembourg, IBBL) each presented automated processing systems that are in use at their facilities. Dr. Zimmermann developed systems for the European Bank for induced pluripotent Stem Cells (EBiSC), including a mobile unit for sample collection and initial processing; an Askion GmbH cryoprotective hood system to maintain a cold chain throughout handling; and a vitrification (flash freezing) protocol to maximize cell preservation.¹² He pointed out that implementing automation is very time consuming and estimated that it might take 2 to 3 years for the *All of Us* Biobank to have a completely implemented automated protocol. He noted that while automation may not lower the cost or increase the speed of cell isolation compared with manual processing, automated systems are both scalable and transferable—important considerations for a project of the magnitude of the *All of Us* Research Program.

The system implemented at IBBL in Luxembourg and presented by Mr. Ammerlaan employs CPTs and a Tecan liquid handling robot to draw off and aliquot the plasma, and recover the cell layer after centrifugation. The robot centrifuges the cell layer, resuspends it in a cold cryoprotective agent, and transfers it into a cryovial. Cryovials are manually placed in a controlled rate freezer then transferred to liquid nitrogen for long-term storage. Up to eight tubes can be processed in a 70-minute cycle. During 2016 the IBBL processed 920 samples following this method. The team at IBBL has validated a number of key variables for automated isolation of viable PBMCs.¹³

In the subsequent discussion, there was a general consensus that it would be possible to develop an automated solution to permit isolation and preservation of viable cells from at least part of the *All of Us* cohort. The group recommended that the starting material have as many nucleated cells as possible. The

consensus was to collect at least four 8 mL whole blood tubes, which ideally will yield 32–40 million nucleated cells. The group concurred that cells should be preserved at a concentration of 5–10 million/mL for optimal preservation, so starting with four 8 mL whole blood tubes should yield as many as four aliquots of preserved cells. The addition of four additional whole blood tubes, however, could present issues given the total number of blood tubes collected for the *All of Us* Research Program. There was also good consensus that the use of CPTs is a good starting point, as there is currently marketed equipment to detect the PBMC layer in the tube after centrifugation. However, several participants advocated the use of SepMate or LeucoSep tubes that do not require a detection device to automatically draw off the PBMC layer because these tubes contain an insert barrier that separates PBMCs from the red blood cells, granulocytes, and density gradient media. It is likely that there will be significant variability in cell concentration among participants, based on their health status and age, so it was recommended that the workflow should include cell counting. This could create a challenge to the process to automate the workflow pipeline.

Conclusion

In large measure, biomarker discovery has not yielded many useful biomarkers to improve diagnosis, prognosis, or overall health outcomes,¹⁴ suggesting that it is important to explore other research avenues for biomarker discovery. One such direction is the use of viable cells from well-characterized participant cohorts to carry out functional studies. Observing a cell's response to stimuli or conditions will allow researchers to better understand an individual's response to those stimuli or conditions. In addition, functional assays offer many more potential outputs that might serve as useful, robust biomarkers, such as the pattern of translation in response to a treatment. These molecular events observed in viable cells can be related to the participant's medical history, environmental or medication exposures, genetic factors, information provided by participant, and data provided by mobile technologies.

The *All of Us* Research Program is the ideal cohort in which to collect viable cells for future functional testing, *because it will be large and diverse*, with rich accompanying phenotypic data.³ In addition, as a single cohort study with uniform protocols and central processing, it should provide a high level of standardization for the samples, yielding a valuable collection with well-characterized preanalytical variables. While the technology to perform functional single-cell analysis is constantly improving and increasingly able to work with smaller number of cells, it is important to collect and preserve these cells upon enrollment to allow meaningful future research that can be correlated with future health outcomes. However, accomplishing these goals will require a robust highly automated system with a sufficient throughput to manage the anticipated number of participants collected over time.

Acknowledgments

We are grateful to those investigators who participated and contributed to this workshop and for the support from the *All of Us* Research Program to conduct the workshop.

Author Disclosure Statement

No competing financial interests exist.

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