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Greta Babakhanova
Carl G. Simon, Jr.
Eugenia Romantseva
Biosystems and Biomaterials Division
Material Measurement Laboratory

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NIST Author ORCID iDs
Greta Babakhanova: 0000-0002-5027-5673
Carl G. Simon, Jr.: 0000-0001-5209-3577
Eugenia Romantseva: 0000-0003-0929-7966

Contact Information
greta.babakhanova@nist.gov

Abstract

Biofabricated tissue-engineered constructs have the potential to transform personalized medicine. However, characterizing these constructs post-fabrication and throughout preclinical use remains challenging. On December 1, 2022, the National Institute of Standards and Technology (NIST) held a one-day, virtual workshop on measurement needs for biofabricated constructs that contain cells. The workshop focused on metrology for the structure of the constructs, cell viability in the constructs, and functional capacity of the constructs, with the aim of enabling future research directions, standards development, and the adoption of these constructs for clinical use.

The workshop convened over 180 participants and represented academia (28%), government (37%), industry (25%) and non-profit (10%) sectors. The attendees focused on discussing and identifying measurement needs for characterizing biofabricated, tissue engineered medical products (TEMPs) for clinical applications. Expertise of participants included tissue engineering (34%), biomaterials (18%), additive manufacturing (16%) and sensors (11%), among others (22%). The cells and tissues being targeted by participants included musculoskeletal (29%), mesenchymal stem cells (14%), cardiac (9%), fat (8%) and endothelial cells (8%), among others (34%). A survey to query participants about which measurements were most in need of improvement yielded the following results: potency (18%), cell viability (14%), structure (13%), pH-O_2-metabolites (10%), and mechanical properties (8%), among others (39%).

The workshop revealed that the field of biomanufacturing finds itself in a nascent stage characterized by destructive, labor-intensive methods. The unanimous call for non-destructive, accurate viability and functionality characterization highlights a pivotal need for innovation. Simultaneously, there is a mounting demand for standardized testing, manufacturing parameters, and reference materials, coupled with application-specific standards for different cell types and manufacturing processes. The establishment of collaborative consortia is advocated to foster knowledge sharing and effective integration of technologies. The identified challenges in manufacturing consistency underscore the pressing need for repeatable, reproducible, robust measurement techniques, and interdisciplinary collaborations in biomanufacturing. Additionally, the pivotal role of systems capturing diverse measurements, the preference for minimally invasive sensors, and efforts towards miniaturization of clinical technologies for lab use collectively propel the field towards a progressive and multimodal approach for tissue characterization. The unanimous support for the collection, validation, and standardization of reference data, with the proposal of a central data portal, signifies a concerted effort towards enhancing the reliability and accessibility of crucial information in the field. The insight from the workshop documented in this report can help guide future work on the development of measurements and reference materials to facilitate the biofabrication of tissue engineered medical products.

Keywords

Biofabrication; regenerative medicine; tissue engineered medical products (TEMPs); standards; measurement needs; workshop report.

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1. Workshop Goals and Organization

The workshop on Measurement Needs for Biofabrication of Tissue Engineered Medical Products aimed to identify and discuss the challenges and opportunities of measuring biofabricated constructs for clinical use. These constructs, which combine cells and biomaterials, have the potential to revolutionize personalized medicine, but they require reliable and robust characterization methods to ensure their quality, safety and efficacy. The workshop focused on three key aspects of biofabrication metrology: (1) the structure of the constructs, (2) the viability of the cells within them, and (3) the functional capacity of the resulting tissues. The workshop also explored how measurements in these three areas could facilitate future research, enable new standards development, and help clinical adoption of biofabricated tissue-engineered products.

Tissue engineering involves use of cells, scaffolds, biomaterials and biomolecules to create constructs that can be implanted into patients to augment or restore tissue and organ function. Tissue engineering is an emerging technology that may one day be able to provide bioengineered replacement organs such as kidney or heart. Several TEMPs have been approved for human use, such as the following examples: (1) Apligraf, which consists of allogeneic fibroblasts in collagen for treatment of diabetic ulcers, (2) MACI, which consists of autologous chondrocytes in a collagen membrane for treating knee cartilage defects, (3) Rethymic, which consists of allogeneic processed thymus tissue for treating congenital athymia, and (4) Stratagraft which consists of allogeneic keratinocytes in collagen for treating burns [1].

A pre-workshop survey (Section 3) was used to identify the most relevant topics for discussion. As a result, the agenda included three main sessions: cell viability, cell phenotype, and tissue characterization. These three topics cover most properties that need to be characterized for TEMPs. Cell viability is a fundamental cell attribute that any product containing live cells will need to assess. Cell phenotype is specific to the clinical indication and is key to the function of a TEMP. Tissue structure is a defining aspect of a biofabricated construct where the 3D placement of the components provides added functionality to the TEMP. Each session featured presentations from industry, academia, and government experts, followed by panel discussions.

After the main sessions, the participants were divided into breakout groups to address specific questions related to one of the following topics: (1) potency and phenotype measurements, (2) cell viability measurements, (3) measurements of construct structure, (4) pH, O_2, and metabolite measurements, (5) mechanical property measurements, (6) sterility measurements, (7) measurements of cell distribution in constructs, (8) measurements of raw materials, (9) tissue mimics, and (10) tissue reference data.

2. Findings and Recommendations

2.1. Findings

- The biomanufacturing field is nascent and largely relies on destructive, labor-intensive methods for structural and functional characterization of TEMPs.
- There is a lack of reference materials that can mimic the structural complexity and functionality of native tissue.
- A common repository for collecting, validating, and storing characterization data from both engineered tissue products and native healthy tissues will aid in the development of tissue reference standards and in silico tissue models.
- Standardized material data sheets with validation specifications for raw materials will aid in material selection and improve the reproducibility of TEMPs.
- Improved sterilization methods that treat the full geometry of TEMPs without affecting structure and function are needed to advance the adoption of TEMPs for clinical applications.
- The formation of a TEMPs consortium would address measurements, consensus standards, and technologies needed to increase confidence and adoption of TEMPs beyond the bench.

2.2. Recommendations

- Prioritize the development of instrument platforms that leverage miniaturization and integration of multimodal technologies for comprehensive, non-destructive, and real-time measurements of TEMPs.
- Encourage the adoption of population-level measurements, rather than single-cell or subsample measurements to ensure comprehensive characterization and enhance reproducibility.
- Integrate in-line process measurements for real-time characterization of raw materials throughout the TEMPs manufacturing pipeline.
- Develop a common lexicon with community accepted definitions, for example, for potency, efficacy, tissue mimic, and tissue reference data to advance the development of measurements, standards, and technologies for improved reproducibility.
- Build a framework for case studies and validation of TEMPs by multiple laboratories to facilitate reproducibility.
- Develop a repertoire of community accepted protocols and best practices, for example, for imaging of biomaterials and bioinks, data collection and reporting, and tissue preparation and preservation to aid reproducibility and adoption of TEMP technologies.

Table 1: Summary of key points from the workshop.		
Key Points		**Summary**
Challenges in Biomanufacturing		• Relies on destructive, labor-intensive methods • Lack of reference materials • Measurement reproducibility issues
Solutions Needed	Collaboration & Standardization	• A common repository for data collection • Standardized material data sheets • Improved consistency in TEMPs manufacturing • Development of community-accepted protocols and best practices • Formation of TEMPs consortium • Development of a common lexicon
	Technological Advancements	• Improved measurements for cell viability, tissue function and tissue structure • Integration of multimodal technologies for comprehensive, real-time TEMPs measurements • Development of population-level measurements • Improved sterilization methods • Integration of in-line process measurements

3. Pre-workshop Survey

The Standards Coordinating Body (SCB) disseminated the pre-workshop survey to SCB and ARMI|BioFabUSA stakeholders, the Society for Biomaterials Tissue Engineering Special Interest Group, and the list of stakeholders compiled by the workshop organizing committee. The survey was active from August 1 to August 23, 2022. The results of the survey informed the discussion topics and structure of the workshop. There were 42 respondents who represented academia, industry, government, and non-profit sectors as shown in Fig. 1. The self-identified areas of expertise of the respondents is illustrated in Fig. 2.

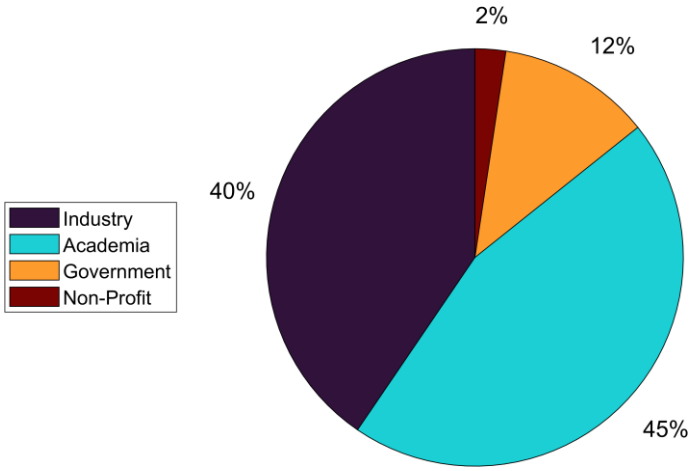

Fig. 1. Demographics of pre-workshop survey respondents.

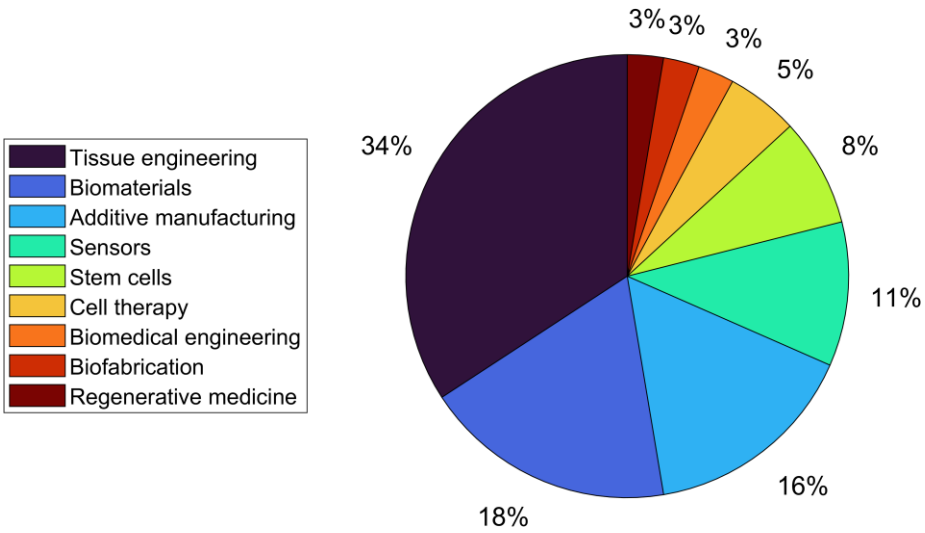

Fig. 2. Area of expertise of the pre-workshop survey respondents.

The survey participants identified four areas for improvement that are most crucial for enabling the clinical adoption of biofabricated constructs: (1) potency/phenotype, (2) cell viability, (3) structural measurements, and (4) monitoring pH/O_2/metabolites (Fig. 3).

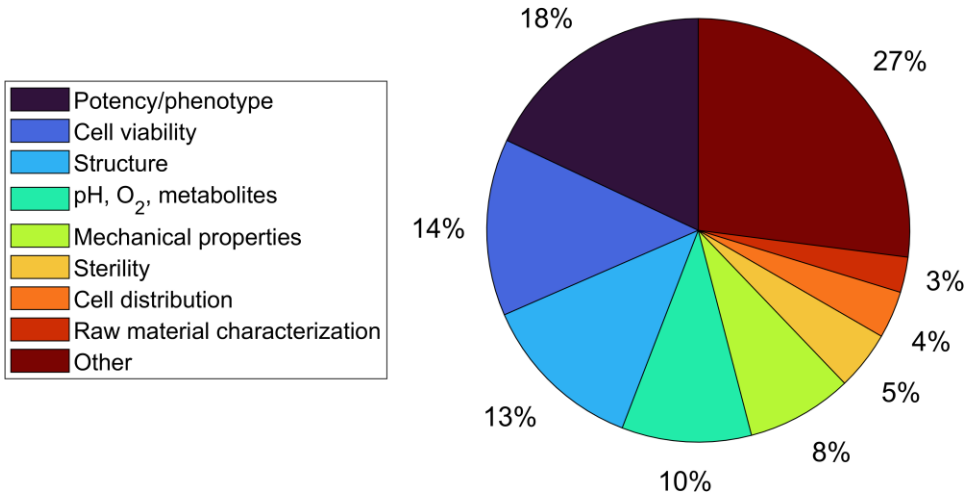

Fig. 3. Measurements most in need of improvement.

Regarding measurement variability, stakeholders highlighted four measurement areas where achieving reproducibility across different sites (*e.g.*, laboratories) and users remains challenging: (1) potency or phenotype, (2) mechanical properties, (3) structure, and (4) cell viability for biofabricated constructs (Fig. 4).

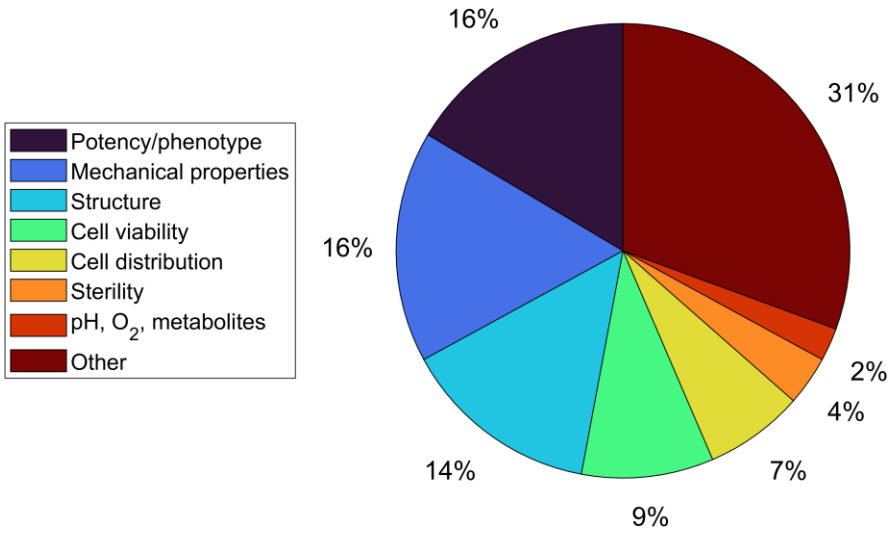

Fig. 4. Measurements difficult to reproduce.

In response to the question regarding essential reference materials for calibrating measurements of biofabricated constructs, the key requirements included (1) reference scaffolds, (2) tissue reference data, (3) tissue mimics, (4) reference biomaterials, and (5) documentary standards (Fig. 5).

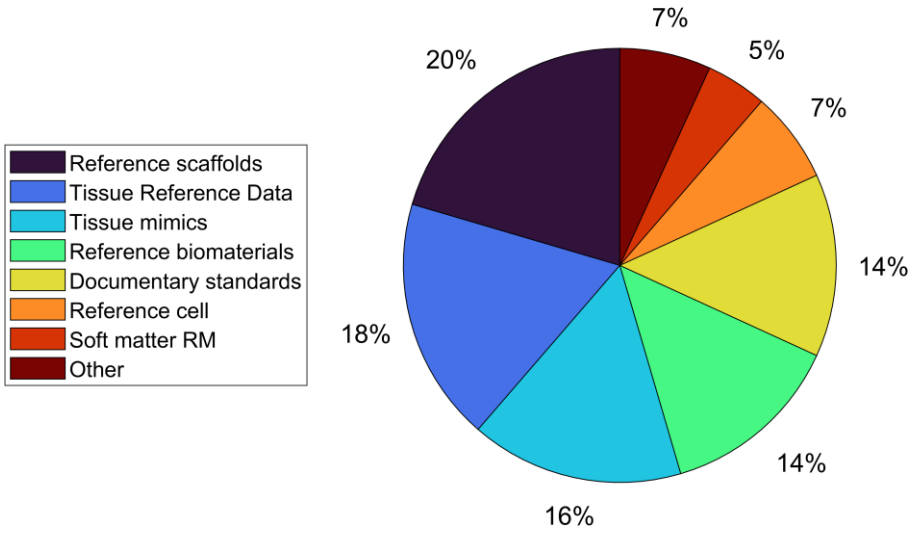

Fig. 5. Needed reference materials.

When asked about the target application(s) and clinical indication(s), the top four responses stakeholders provided were, (1) musculoskeletal, (2) cardiac or heart valve vasculature, (3) skin or wound, and (4) mesenchymal stem cells (MSCs) (Fig. 6).

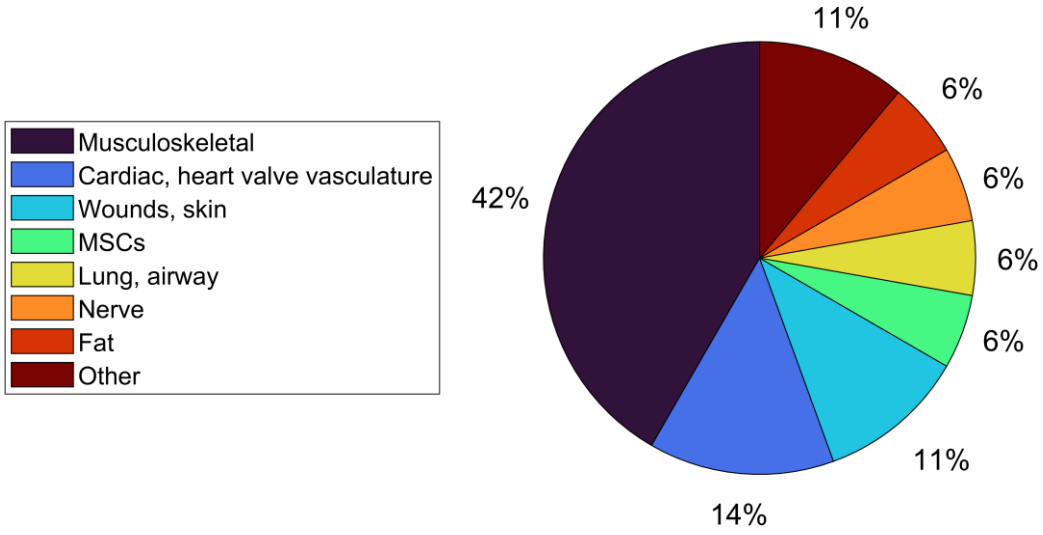

Fig. 6. Target application(s) and clinical indication(s).

Regarding target cell or tissue type(s), stakeholders identified the following top four responses: (1) musculoskeletal, (2) mesenchymal stem cells, (3) cardiac or heart valve, and (4) fat (Fig. 7).

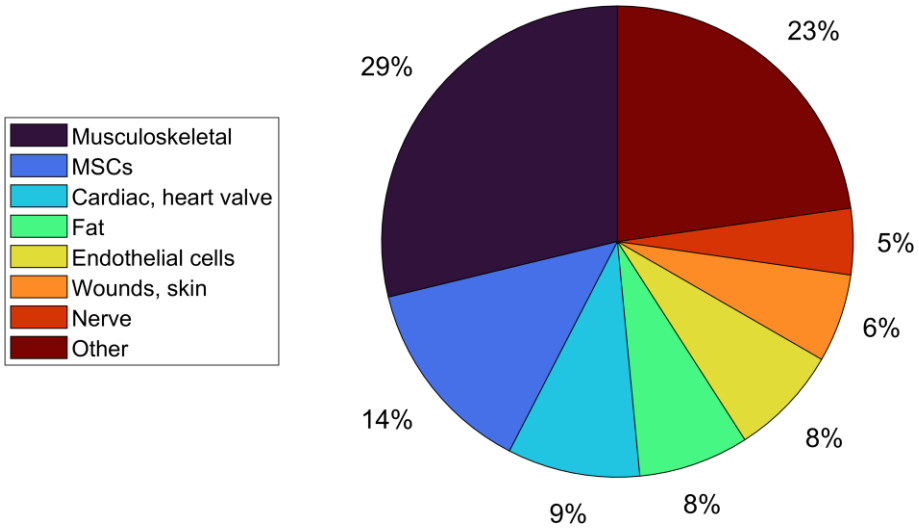

Fig. 7. Target cell or tissue type(s).

When asked about potential topics for future workshops on biofabrication, the top three suggestions from stakeholders were (1) characterization throughout biofabrication, (2) best practices for biofabrication, and (3) raw materials for starting stock (Fig. 8).

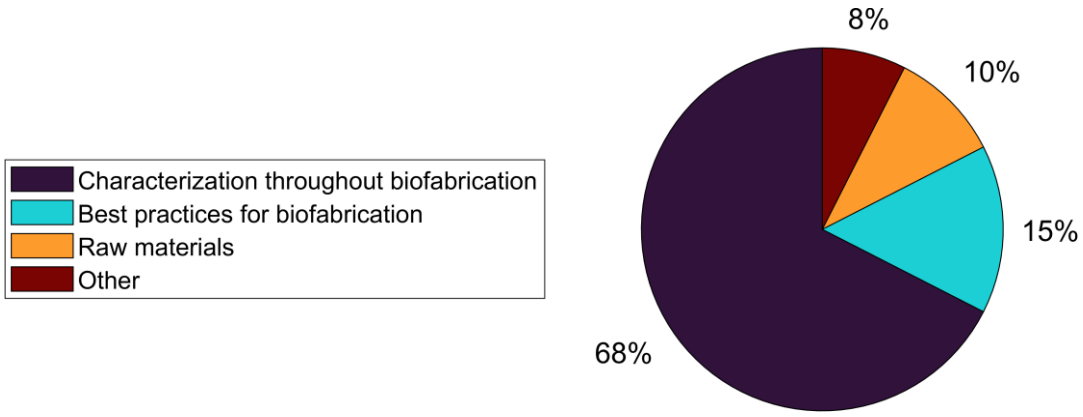

Fig. 8. What topics could be addressed in future workshops on biofabrication?

4. Main Session

4.1. Opening Remarks

Dr. Sheng Lin-Gibson, Chief of NIST's Biosystems and Biomaterials Division (BBD), commenced with the mission of NIST and BBD, highlighting how existing NIST programs aim to support the growing regenerative medicine industry. Notably, NIST's Regenerative Medicine and Advanced Therapies Program encompasses cell and gene therapy, as well as tissue engineered products. The program aims to take a holistic view on all of the aspects that are required for commercialization including the development of new measurement capabilities and methods needed for a broad range of starting materials, products, and critically needed reagents. Dr. Lin-Gibson also highlighted new capabilities at BBD to address biofabrication challenges, including biometrology (*e.g*., biomolecular, genomics/multi-omics, or cell measurements), engineering biology (*e.g*., cell line engineering, genome editing), and core platforms (end-to-end bioprocessing, automation). She emphasized the efficacy of public-private partnerships that address pre-competitive challenges through NIST-led consortia (*e.g*., Genome in a Bottle Consortium, Genome Editing Consortium, Flow Cytometry Standards Consortium, and Rapid Microbial Testing Methods Consortium). She emphasized the value of standards in industry and listed the various types of standards, including reference materials, reference data, documentary standards, and calibration services, all of which are intended to hasten development, manufacturing, and product approval.

4.2. Plenary Session

Dr. Jennifer H. Elisseeff, *Johns Hopkins University*

Dr. Jennifer H. Elisseeff gave a talk on manufacturing complex biologics for regenerative immunology. She shared her insights on translating technologies with multiple and unknown modes of action, and the obstacles they encountered along the way. She highlighted the need for manufacturing standards, especially for cells.

She discussed two technologies for tissue repair developed in her group: (1) a synthetic hydrogel and (2) an adhesive made from a biopolymer. She explained how manufacturing consistency of the biopolymer posed a challenge during manufacturing. She demonstrated how clinical translation informs new research directions and fosters public-private partnerships.

The translation of her group's technologies led Dr. Elisseeff to explore regenerative immunotherapies for tissue repair. Currently, her group investigates the immune response to injury and biomaterials, with a special focus on the adaptive immune system and its role in repair processes. She suggested that the immune system is therapeutically accessible and is the right target for regenerative medicine.

Dr. Elisseeff concluded by explaining that injuries and biomaterials can both affect the body beyond their immediate location. Specifically, extracellular matrices (ECMs) are complex biologics that require careful measurement and regulation to ensure their safety and

consistency for patient use. She also expressed the need for combination products for aging and personalized medicine.

4.3. Session 1: Cell Viability Presentations

4.3.1. Non-invasive, Non-contact, Real-time Monitoring of Cells Within Bioreactors by Direct Imaging With Optical Coherence Tomography

Dr. Naresh Menon, *CEO, ChromoLogic*

The optical coherence tomography (OCT)-based technology, presented by Dr. Naresh Menon, enables imaging of cells in motion within a bioreactor [2]. The image is generated by the cell's own motion, without any external intervention. This technique is non-invasive and does not pose any risk of contamination or require probe insertion into the bioreactor. The OCTiCell system is designed for automated and continuous measurements of cell concentration, viability, and size, and supports remote monitoring and operation. It does not use consumables and can be adapted to various bioreactor types (*e.g.,* glass or plastic), including small scale bioreactors, such as shaker flasks, that are incompatible with other monitoring methods. It can also perform multi-reading monitoring by connecting multiple probes to a single system. ChromoLogic is actively seeking applications that can leverage the unique advantages of OCTiCell.

4.3.2. Nondestructive Cell Viability Assessment Using Oxygen Imaging

Dr. Mrignayani Kotecha, *President, O2M Technologies*

Dr. Mrignayani Kotecha demonstrated a novel noninvasive technique for measuring cell viability in 3D scaffolds [3]. She explained the drawbacks of existing cell viability methods, such as their destructive nature, output data that relies on arbitrary intensity values, intracellular reagent uptake that may affect the results, and lack of spatial information. She argued that an ideal method should preserve the structure and function of the cells and the biomaterial, provide direct and quantitative measurements in SI units, and have a large penetration depth. She introduced the O2M electron paramagnetic resonance oxygen imaging (EPROI) technology [4], which uses an oxygen-sensitive spin probe and EPROI to visualize the oxygen distribution and consumption in the tissue construct. She showed that this technique is non-toxic and suitable for *in vitro* and *in vivo* applications.

Dr. Kotecha demonstrated how to visualize oxygen diffusion in biomaterials and estimate the time required for full oxygenation of a 3D construct. The method is quantitative and measures the amount of oxygen in the system in moles. It can obtain accurate oxygen images with a resolution of 1mm Hg. Moreover, their technique does not require cryogenic cooling and can support physiological conditions during measurement acquisition. The method can penetrate 6-8 cm and can be applied to any 'cell+scaffold' constructs. Their enhanced pO_2 imaging enables

cell viability assessment in tissues of any size and shape, and could be a valuable predictor of the effectiveness of TEMPs.

4.3.3. Optical Coherence Tomography Imaging for Label-free Measurement of Cell Viability in Scaffolds

Dr. Carl G. Simon, Jr., *National Institute of Standards and Technology*

Dr. Carl Simon demonstrated a rapid and label-free method for imaging large volumes of samples (1 mm^3 in 2.5 min) to quantify the viability and distribution of cells within scaffolds using OCT [5]. OCT imaging was used to assess a model system consisting of a polysaccharide-based hydrogel seeded with human Jurkat cells. He compared four scenarios: (1) hydrogel with live cells, (2) hydrogel with heat-shocked dead cells, (3) hydrogels with fixed dead cells, and (4) hydrogel without cells. OCT images revealed time-dependent changes in the refractive index (RI) within live cells that were due to intracellular movement of organelles, referred to as speckling patterns. These patterns were absent in hydrogels without cells or with dead cells. The changes in speckle patterns were used to generate live-cell contrast by image subtraction where objects with large changes in RI were binned as live cells. Additionally, the 3D distribution of live cells was mapped within a hydrogel scaffold to evaluate their uniformity across the volume. This method holds promise in the evaluation of TEMPs.

4.3.4. Cell Viability Panel Discussion

Moderator: Dr. Alicia Henn, *BioSpherix*
Panelists: Dr. Naresh Menon, *CEO at ChromoLogic*
Dr. Mrignayani Kotecha, *President at* O2M Technologies
Dr. Carl G. Simon, Jr., *Biologist at NIST*

In the panel discussion, speakers emphasized crucial steps required to transition biofabrication from the state-of-the-art to the ideal manufacturing process for organ and limb replacements. One of the key points highlighted by the panelists was the need for comprehensive and orthogonal datasets that instill confidence in the final product. By comparing such datasets, we can identify the thresholds of variability inherent in biological systems, enabling us to better understand and address the sources of variability. To reduce variability in the manufactured products, there is a call for refinement of measurement techniques, both at the tissue- and organ-level. Creating platforms that capture diverse, orthogonal measurements for known processes or bioreactors is an essential aspect of advancing our knowledge at different stages of biomanufacturing. Making the resulting data publicly available would further facilitate the exploration of correlations and progress the field.

The overarching theme emphasized by the panelists was the need for accurate and trustworthy data. Such data is pivotal in driving innovation and ensuring the safety and efficacy of the manufactured products during clinical studies. Another critical aspect was the need to establish

better control over the microenvironment at the beginning of the manufacturing process. This improved control is key to achieving reproducibility.

The panelists stressed the significance of fostering interdisciplinary collaborations. Bringing together experts from diverse fields can lead to novel insights and innovative approaches that can significantly advance the biofabrication field. In conclusion, the panel discussion highlighted the importance of data, reproducibility, non-invasive measurement techniques, and interdisciplinary collaborations for advancement of biomanufacturing. By addressing these areas, we can accelerate progress and ultimately improve the lives of many in need of such advancements.

4.4. Session 2: Cell Phenotype Presentations

4.4.1. Advancing Drug Discovery with Biofabricated 3D Tissue Models

Dr. Marc Ferrer, *National Center for Advancing Translational Sciences*

Dr. Marc Ferrer discussed how 3D organotypic models can be used as predictive tools for drug discovery and development. He emphasized the importance of quantitative measurements for validating the biological and physiological relevance of the models and for developing functional phenotypic assays for drug testing. He also highlighted the need of ensuring the predictability, reliability, and robustness of 3D organotypic models for early discovery and pre-clinical drug development.

His team developed a versatile suite of biofabricated 3D tissue models with different levels of physiological complexity. For example, he showed lung epithelial tissue models with varying degrees of complexity. Dr. Ferrer demonstrated the physiological validation of each tissue using various techniques, such as histology, immunofluorescence, single-cell RNA sequencing, mass spectrometry, OCT, metabolomics and proteomics. However, he noted that these techniques are low-throughput, so his team is working on developing functional assays that are compatible with high-throughput screening (HTS), such as cell viability, barrier function, multiplexed cytokine secretion, and 3D high content imaging.

Dr. Ferrer presented several studies, including the pathology of respiratory viruses using the air-liquid-interface lung epithelial tissue model [6]. However, his team found that the assays for validating the tissues were not suitable for HTS. Therefore, they switched to fluorescence biosensors and delivered them to the cells via adeno-associated virus transduction [7]. The biosensors enabled them to quantify various cellular responses, such as calcium release and neurotransmitter release of dopamine and glutamate. They also employed channelrhodopsins to manipulate neuronal activity with optogenetics and modulate the activity of the neuronal circuits. Dr. Ferrer's team aims to use these biosensors for compound testing with bioprinted neuronal hydrogel-based models. Their ultimate goal is to create functional neuronal circuitry that can be controlled by optogenetic stimulation.

4.4.2. Protein Sensing

Dr. Marcie Black, *Advanced Silicon Group*

Dr. Marcie Black began her presentation by highlighting the importance of sensing in TEMPs. She explained that sensing is essential for every stage of the biomanufacturing process. She focused on how protein sensing can be used to monitor cell growth by detecting protein biomarkers to ensure the quality and consistency of the raw materials and optimal use of growth factors, which are a major cost factor for TEMPs.

Dr. Black acknowledged that non-invasive sensors do not exist, and instead her work focuses on developing minimally invasive sensors that minimize interference with their applications. One way to achieve this is to reduce the size of the biosensor and the amount of sample solution required. LightSense uses silicon sensors that can be scaled down to very small sizes, allowing reduced sample volumes and increased throughput. These sensors use nanowires that have a high surface area to volume ratio, making them sensitive to their surroundings and enabling them to detect very low levels of target proteins in the solution because protein binding is enhanced on the nanowire surface.

According to Dr. Black, biosensors that perform electrical measurements have several advantages over optical measurements. For example, they are more sensitive, more cost-effective, and more quantifiable. Dr. Black identified three promising technologies that could meet the market demand for TEMP sensing: (1) electrical measurements, which are preferred over optical ones for the reasons mentioned above, (2) silicon, which has a wide knowledge base in the semiconductor industry and can be scaled to lower the cost, and (3) nanowires, which have a high surface area to volume ratio that enhances their sensing capabilities. Improving protein sensing technology will have positive impacts on many fields that rely on TEMPs.

4.4.3. Non-invasive Quantitative Live Cell Imaging

Dr. Kersti Alm, *Phase Holographic Imaging*

Quantitative phase imaging (QPI) is a technique that allows for label-free and minimally invasive cell phenotypic measurements. Dr. Alm discussed how holographic imaging, a form of QPI, can be used to monitor cell health, movement, morphology, viability, and behavior in real-time. She argued that QPI is becoming a widely accepted method for cell analysis [8].

She also demonstrated their HoloMonitor system, which produces holograms that contain information about cell thickness in each pixel of the image. These holograms can be reconstructed to display the sample topography in 3D. Various morphological parameters can be extracted from the images. The time-lapse imaging does not damage the cells during data acquisition. The technology has numerous applications including cell tracking, kinetic cell proliferation, cytotoxicity, and wound healing. According to Dr. Alm, QPI techniques have gained recognition as viable approaches for cell quality analysis.

4.4.4. Cell Phenotype Panel Discussion

Moderator: Dr. Jeff Halpern, *University of New Hampshire*
Panelists: Dr. Marc Ferrer, *National Center for Advancing Translational Sciences*
Dr. Marcie Black, *Advanced Silicon Group*
Dr. Kersti Alm, *Phase Holographic Imaging*

The panel's primary focus was to address the challenges associated with adapting their technologies for broader applications, comparing 3D cultures with traditional 2D cultures, and developing sensors with high reproducibility. The consensus among the participants was that no single technology can offer a comprehensive solution. For instance, while optical detection provides real-time and cell-friendly insights, it may not always yield the quantitative results required. Therefore, a combination of techniques is often necessary to obtain a more comprehensive understanding.

Participants suggested that increased collaboration and knowledge sharing among developers would be highly beneficial. The formation of a consortium was proposed to bring together experts and explore the most effective ways to integrate and combine technologies to enhance the final product.

The participants recognized the importance of tailoring technologies to specific applications and stages of product development while carefully assessing their impact on biology. They also discussed the complexities of understanding 3D cell cultures compared to 2D cultures. Dr. Alm mentioned that their digital holography approach, initially designed for analyzing 2D cultures, is now being adapted for 3D applications such as spheroids. Dr. Black explained that their technology inherently operates in 1D, and achieving a 3D perspective involves sampling at various locations within the sample. Dr. Ferrer highlighted that biofabricated tissues ranging in diameter from 100 µm to 200 µm are suitable for confocal microscopy due to their clarity, whereas spheroids or organoids tend to become dense and dark, necessitating the development of clearing protocols and immunofluorescence imaging reagents.

The participants stressed the importance of developing nondestructive and reproducible technologies for product assessment. Consistency and repeatability were identified as significant challenges for developers. Addressing the issue of reproducibility becomes even more complex when working with primary patient cells, as their behavior can vary between donors and lots. Currently, the field is immature in terms of what can be effectively detected, and many existing approaches are destructive and require rigorous validation. Therefore, the community would greatly benefit from a range of technologies that enable quality control without the need to sacrifice expensive tissues. Low-throughput yet noninvasive methods hold promise for assessing tissue maturation while maintaining tissue integrity.

4.5. Session 3: Tissue Characterization Presentations

4.5.1. Dielectric Spectroscopy for In-line Monitoring of Engineered Tissue Constructs

Dr. Rohan Shirwaiker, *North Carolina State University*

Dr. Rohan Shirwaiker provided an insightful overview of dielectric spectroscopy (DS) as a valuable tool for nondestructive quality monitoring in tissue engineering [9]. He leads the 3D Tissue Manufacturing Group, which delves into various aspects of scalable manufacturing technologies for engineered tissues, with a strong emphasis on quality engineering.

Methods for quality assessment in the field of tissue engineering originated from measurements of 2D and suspension cultures, and they are now being adapted for use in 3D constructs. Unfortunately, these conventional methods often involve offline, destructive, and labor-intensive procedures. Innovative approaches, such as the fabrication of sacrificial surrogates, are required to overcome these limitations, develop manufacturing schematics for TEMPs, and adapt existing quality assessments for 3D cultures.

Dr. Shirwaiker's team leverages DS for in-line monitoring of bioprinted constructs within a bioreactor system. He highlighted three distinct case studies to illustrate the effectiveness of this technique.

First, DS enables the quantifiable monitoring of process deviations during bioprinting. This means that after printing a construct, one can immediately assess how the process performed and whether there were any deviations from the optimal settings.

Second, the technique proves valuable when culturing a bioprinted construct in a bioreactor, as it can detect malfunctions by measuring significant drops in delta permittivity (a measure of how the electric polarizability of a sample changes under specific conditions). This drop correlates with changes in glucose concentration, indicating perfusion issues and deviations in the ongoing process.

The third example showcased how DS can be employed to assess personalized tissue constructs. For instance, in the case of printing a knee meniscus, it can be used to evaluate the performance in different parts of the construct after printing each layer and over time. This allows for the assessment of localized behavior and changes in the delta permittivity, which provides insights into cell viability. Advanced data analytics methods, including machine learning-driven spectral analysis, decipher subtle differences that may not be immediately apparent.

Dr. Shirwaiker emphasized that relying solely on scalar delta permittivity metrics cannot provide information about cell types or variations in cell volumes. To make informed decisions, a comprehensive analysis of the entire spectra is essential. This approach can predict the onset of differentiation and the specific lineage along which stem cells are differentiating, demonstrating its practical applicability for quality monitoring in tissue engineering.

4.5.2. In Situ Volumetric Imaging and Analysis of 3D Bioprinted Constructs Using Optical Coherence Tomography

Dr. Adam Feinberg, *FluidForm*

Dr. Feinberg emphasized the pressing need for in-situ 3D imaging and validation to ensure dimensional accuracy and detect any errors during the bioprinting process. His team developed the Freeform Reversible Embedding of Suspended Hydrogels (FRESH) [10]. This technology facilitates the printing of cells within soft hydrogels embedded in another hydrogel, which offers physical support to prevent deformation and collapse of the structures, while maintaining an aqueous environment.

The support gel used is a yield stress material, allowing the printing needle to move through it while the support gel flows around the needle. Importantly, the gel uniformly supports the bioink during printing while it undergoes gelation. This printing process occurs at room temperature, and later, the support gel can be melted to non-destructively retrieve the printed construct. The support bath consists of gelatin microparticles in an aqueous phase. It enables traditional layer-by-layer printing, non-planar layer-by-layer printing, and freeform architecture, effectively eliminating the influence of gravity in the bioprinting process. This approach is highly versatile, compatible with a wide range of bioinks, crosslinking mechanisms and support bath materials. For instance, collagen can be printed with a pH change, alginate with ionic crosslinking, fibrin with enzymatic crosslinking, and methacrylated hyaluronic acid with photo crosslinking.

Dr. Feinberg's team leveraged FRESH to create vascularized and perfused tissues that could be characterized and quantified during the fabrication process. They designed a series of bioreactors tailored for *in-vitro* culturing of soft biological tissues. Structural validation was a critical concern, particularly when dealing with multiscale fluidic networks where even a single inclusion or defect could disrupt the entire system. To address this, they integrated the OCT scan head directly with the bioprinting system and used OCT to non-destructively assess the print fidelity of the constructs in real time during the printing process [11]. This integration enabled the detection of errors within the print. Advanced error detection capabilities included identifying blocked channels, excessively thick or thin walls, defects caused by bubbles, and over-extrusion.

Dr. Feinberg stressed the critical importance of measuring print quality since it directly impacts functional performance. Furthermore, it represents the type of quality control necessary to comply with regulatory agencies, such as the FDA, for clinical translation.

4.5.3. Characterization of Tissue Engineered Medical Products

Dr. Bao-Ngoc Nguyen, *The U.S. Food and Drug Administration*

Dr. Bao-Ngoc Nguyen focused her talk on regulatory considerations for TEMPs, which are considered combination products (*e.g.*, products composed of different categories of regulated articles) under FDA definition 21 CFR 3.2(e). Combination product may include a device-

biologic, biologic-drug constituent, drug-device, or trifecta biologic-drug-device constituent. Constituents are intended for use together in a combination product and they are required to mediate the intended therapeutic effect. These constituents can be physically or chemically combined, or they can even be co-packaged and must be cross-labeled to be considered a combination product. The jurisdiction of a combination product is assigned based on Primary Mode of Action (PMOA) outlined in the regulations (21 U.S.C.§ 503(g)). PMOA is defined as the single mode of action of a combination product that provides the most important intended therapeutic action of the combination product.

Dr. Nguyen further discussed several combination product examples such as cell-seeded scaffolds. During combination product development one needs to consider the cells, the scaffolds, and the combination product as a whole. The regulatory concerns and considerations related to cells include (1) quality of reagents used to prepare cells, (2) cell bank safety testing, (3) use of feeder layer, (4) level of cell characterization, (5) quality of manufacturing facility, (6) aseptic processing, (7) cell viability, and (8) cell stability. The regulatory concerns related to scaffolds include (1) quality of materials used to synthesize scaffold, (2) residual reagents, (3) biocompatibility with cells and tissues, (4) physical strength and integrity, (5) equipment and facility used, (6) stability *in vitro* and *in vivo*, and (7) scaffold sterilization. The regulatory considerations related to combined cell-scaffold products include (1) the impact of cells on the properties of the scaffold, (2) the impact of scaffold on the properties of cells, (3) testing of construct, (4) uniformity, (5) reproducibility, (6) handling at the clinical site, (7) construct stability, and (8) shipping. These considerations usually get summarized in lot release specifications, where expectations are set for how product quality and safety should be measured before it is released or administered to the patient.

Dr. Nguyen also discussed methods for characterization of TEMPs. One method involves using surrogate samples, which are sample products made using identical materials and manufacturing methods as the clinical product, and ideally manufactured at the same time as the clinical product. This could entail making extra samples of product that are intended for testing but not intended for treating a patient. One must provide data that demonstrates that the surrogate is an adequate representation of the clinical product. Another method uses a portion (sub-sample) of the clinical product. For example, unused or extra parts of a clinical product may be used for characterization testing prior to administration. Again, one must demonstrate that portion of clinical product is representative of entire clinical product. When the characterization of cells or scaffold is not feasible without taking the cells off of the scaffold, the separation of cells from scaffold is needed to evaluate cell characteristics and scaffold parameters. In this case, the impact of dissociation of cells from scaffold should be considered. Lastly, sometimes it is unavoidable to utilize portions of the clinical lot or the entire product for lot release testing. As such, lot release testing should be conducted on final product after all manufacturing steps have been completed.

Dr. Nguyen concluded by suggesting that non-destructive methods could be utilized to ensure that product quality attributes are not compromised during final product testing. She also advised that manufacturers seek FDA advice early and throughout the entire development process of the product.

4.5.4. Tissue Characterization Panel Discussion

Moderator: Dr. Nathan Castro, *Nanochon Inc.*
Panelists: Dr. Rohan Shirwaiker, *North Carolina State University*
Dr. Adam Feinberg, *FluidForm*
Dr. Bao-Ngoc Nguyen, *The U.S. Food and Drug Administration*

Measuring the mechanical properties of TEMPs post-fabrication is inherent to ensuring the quality, safety, and efficacy of products prior to implantation. For example, it is essential to understand the rheological properties of bioink(s) during the fabrication process, as these can significantly impact factors like cell viability and other essential attributes. The panel discussion highlighted that our current approach to working with tissues is rather rudimentary. It primarily relies on conventional, manual manipulation techniques involving pulling and pushing, which lack the sophistication required for nondestructive, in-line procedures. Consequently, there is a need to develop nondestructive, in-line methods to assess tissue properties, eliminating the current practice of relying on expensive and burdensome clinical lots for testing.

Because there is no single method that can provide comprehensive insights of product performance, particularly for products in the developmental pipeline, there is a growing demand for reference data. These datasets need to cover various aspects of the same manufacturing process obtained from different modalities. This presents an excellent opportunity for data scientists to identify correlations between different sets of data and to utilize data effectively during biomanufacturing. For example, larger organizations can play a crucial role in facilitating the collection and analysis of big data to pinpoint critical process parameters, depending on the intended use of the TEMP.

Technology transfer from the benchtop in academic settings to an integrated, commercial manufacturing workflow that generates the necessary data remains a significant challenge. The introduction of nondestructive technologies becomes especially critical for the sustainability of the business model. The economic feasibility of discarding a significant portion of products due to testing costs must be reevaluated. As the biofabrication field rapidly evolves, more complex applications will necessitate more sophisticated techniques for product analysis.

It is valuable to integrate capabilities for both imaging the structure and measuring functional aspects of a product, such as cell viability. Viability is a concern not only in thick 3D structures post-fabrication but also during the fabrication process itself. The convergence of multiple technologies onto a single platform for nondestructive characterization of a TEMP is not only highly relevant but also a crucial reflection of the field's advancing trajectory.

5. Breakout Session

Each breakout session was given the same questions to guide the discussion. Each group was asked the following: (1) identify the existing measurements that were discussed in that particular breakout session, at the state-of-the-art, (2) identify the limitations of some of the current approaches. Are they non-destructive or minimally destructive? and, (3) list new non-destructive measurements that are needed to move the field forward. The following sections summarize each discussed measurement need.

5.1. Potency & Phenotype Measurements for TEMPs

Discussion Leader: Dr. Richard McFarland (Advanced Regenerative Manufacturing Institute)

One of the challenges faced by the potency & phenotype breakout session was the lack of a common understanding of the key concepts related to the topic. The participants needed to agree on the definitions of measuring potency, potency test, and efficacy before they could effectively tackle the questions assigned to them.

The terms potency and efficacy are often confused. They refer to different concepts in a regulatory context. Establishing the relationship between potency and efficacy is not straightforward. A potency test measures the strength or effectiveness of the product in relation to its intended therapeutic mechanism of action. The potency assay can only be assessed after the final pivotal clinical study. However, the clinical evidence for the mechanism of action is difficult to establish. Therefore, the question is what to measure before that? Ideally, it should be something that can be measured and that reflects the product's clinical effectiveness (efficacy). Apart from all other challenges, animal models may not accurately reflect the human response, posing another challenge for product development.

The existing measurements for cell potency and phenotype may be product specific. There is no single method that works for all applications. For example, histology is a typical potency test of the skin tissue. Purely non-invasive measurement does not exist, and the measurement itself can affect the cells. For example, the electromagnetic fields used to measure neuronal activity may affect and alter the cells being measured.

The participants suggested the development of the following non-destructive measurements to move the field forward: (1) label-free nanoscale chemical imaging at large volumes (mm^3) over time and (2) quantum sensing and electromagnetic technologies. Additionally, secretome signatures (*e.g.*, proteins, exosomes which may be analyzed with mass spectrometry) or other omics measurements may be powerful and non-destructive measurements of the future.

5.2. Cell Viability Measurements

Discussion Leader: Dr. Mary Clare McCorry (Advanced Regenerative Manufacturing Institute)

The meaning of cell viability varies depending on the system and its application. Therefore, it is important to choose the right measurands for each system, since different cell viability tests have different assumptions. Many of the existing methods use optical colorimetric stains, quantitative microscopy, or biochemistry, and have some drawbacks. They are often invasive, have limited depth of penetration, do not translate to *in vivo* characterization, and do not reflect the functionality of a 3D tissue. The participants discussed the following challenges of current cell viability testing methods: (1) their invasive nature, (2) the scale-up of these measurements, and (3) their inability to capture the heterogeneity of the sample.

The current gold standard for cell viability is the trypan blue (TB) assay. TB method stains dead cells with a ruptured membrane with a blue dye. It is destructive and user-biased since the user interprets which cells are dead based on the degree of blue color. Despite these drawbacks, the TB assay is widely used in the industry.

One of the major challenges in cell viability measurements is finding a representative sample of the entire population. Testing a subsample may not accurately reflect the whole population. Moreover, there may be a difference between the viability state of a single cell and that of the population. A quantitative result from a subsample does not necessarily imply the global viability information of the whole volume. Imaging approaches are also limited by the sample window in a flask, which may not scale well to larger systems such as bioreactors or hyperflasks. Most current technologies fail to work during the scale-up process even for 2D systems, let alone for tissues that are more heterogeneous.

The participants also discussed unique challenges of cell viability assessment in bioinks. Often cells are bioprinted at high density (100 million cells per mL) and need to be stable for a period of time while printing. Current cell viability techniques may work for low density seeding conditions, but may not work at high cell concentrations or are prohibitively expensive (*e.g.*, multiphoton system).

Regarding emerging technologies, the community describes challenges with calibrating these technologies in the presence of changing or heterogeneous populations and backgrounds (*e.g.*, in tissues). For instance, Raman spectroscopy and impedance measurements are hard to calibrate in such conditions. OCT and reflectance measurements are the most direct methods to assess the cell characteristics, but they require further development and refinement.

Session participants recommended multimodal technologies (*e.g.*, a combination of optical and electrochemical methods for 3D characterization) to advance the field. They also emphasized the need for better characterization of products in early stages, prior to animal testing. They proposed to develop a new gold standard for cell viability determination that is non-destructive and robust. This would require orthogonal data sets from current tests, imaging, *etc.*, as well as machine learning, and microenvironment analysis. Moreover, they suggested that emerging technologies in manufacturing should be repeatable, reproducible, amenable to calibration, and have established positive and negative controls to ensure their adoption.

A final point was to keep the KISS principle in mind: “keep it simple and straightforward.” Industry participants stressed the importance of advanced methods in research and development, while also recognizing the value of simpler tests. Representatives from two companies disclosed that their live-cell containing devices had received marketing clearance in the USA. They highlighted the effective use of lactate dehydrogenase and Alamar blue (resazurin) assays for assessing cell viability during manufacturing.

5.3. Measurements of Construct Structure

Discussion Leaders: Dr. Kimberlee Potter (Department of Veterans Affairs) and Dr. Leanne Friedrich (National Institute of Standards and Technology)

Session participants discussed some of the current methods for performing measurements of biofabricated constructs, such as their shape, composition, and microstructure. One of the most common high-resolution methods is confocal microscopy. However, this method has some drawbacks: it is destructive to the sample as it needs fluorescent dyes for labeling, and the imaging depth is limited to the superficial layer of the sample (~100 µm to 200 µm). Light sheet microscopy also has a high resolution, but it needs fluorescent labeling as well. Another common but destructive method is histology, which requires cutting the tissue into thin slices. X-ray imaging can provide high-resolution images of hard constructs (*e.g.*, ceramics, minerals), but it needs a contrast agent to visualize vasculature in large constructs. This may make the constructs unsuitable for implantation and only useful as surrogates. Microcomputed tomography can also provide high-resolution images but only for small organs. Multiphoton imaging does not need labeling and can image deeper layers (up to 1 mm), but it is slow and incompatible with real-time data collection. Among the non-destructive methods, ultrasound and OCT are rapid and minimally invasive, but they have low resolution and cannot provide chemical specificity. 3D holographic imaging is also rapid and non-invasive, but is incompatible with high cell densities.

There is a need to be able to assess large samples with high resolution, which poses both a characterization and a data management problem. Working group participants proposed to develop non-destructive methods (*e.g.*, multispectral modality) and suitable computational tools to obtain composition data. They stressed that it was crucial to find non-destructive characterization techniques for cell-containing products. Additionally, the stakeholders suggested using the concept of digital twins to speed innovation. Digital twins rely on placing sensors in an actual physical sample that provide real time data to an *in silico* model that can be used for computational performance testing.

5.4. pH, O_2 & Metabolite Measurements

Discussion Leaders: Dr. Billyde Brown (Georgia Tech Manufacturing Institute) and Dr. Zeeshan Ahmed (National Institute of Standards and Technology)

Session participants began by summarizing existing methods for measurements of metabolites, oxygen, and pH. This included EPROI for oxygen concentration, MRI, ion-sensitive field-effect transistors (ISFET), optical dyes and fluorescent probes that may be used for pH imaging. Enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance, electrochemical sensors and mass spectrometry may be used to measure metabolites. The capillary electrophoresis-mass spectrometry (CE-MS) is utilized to perform metabolomics and works well with small volumes (a few microliters). DNA barcoding technique that uses DNA tags that bind to specific metabolites and proteins inside cells allows identification of intracellular composition of cells after sequencing.

Next, participants explored the limitation at the state-of-the-art. For example, many techniques have a penetration depth of micrometers, which is insufficient for full product characterization. Also, existing techniques are destructive, since, depending on the application, tissues must be sectioned for analysis and profiling. Optical methods can suffer from signal degradation or photobleaching. Additionally, it is difficult to calibrate pH within bioreactors while maintaining sterility. On top of that, probes can alter the local environment (*e.g.*, electrochemical sensors consume oxygen at the probe tip) affecting the measurement in unexpected ways. Finally, sensors can be limited to certain deployment locations (*e.g.*, in-line, on-chip or offline, in tissue measurements). All these factors may reduce the accuracy of the measurement.

As a result, measurement uncertainty, including repeatability and reproducibility remains a significant challenge in the field. It is challenging to get reproducible results, in part, because the accuracy and precision of the sensors are insufficient. Additionally, because tissues are heterogenous and contain gradients, it is important to ensure that the probe is measuring a location representative of the whole tissue. This is particularly important for co-culture samples where multiple cell cultures are growing at different differentiation and metabolic states. Being able to measure a non-homogeneous cell population via a 3D array or 3D imaging approach would be more informative and could be one path toward reducing measurement uncertainty.

Session participants called for sensor arrays for 3D mapping across a variety of environments. Overall, the field would benefit from moving away from offline measurements and embracing in-situ miniaturized sensors, and in-line and in-situ monitoring. When performing offline measurements, the data at different time points can change. In addition, spatial and temporal resolutions are critical when scaling up sensor technologies for broader deployment and extensive monitoring across diverse environments. To advance measurements of metabolites, oxygen, and pH, participants recommended exploring technologies, such as entangled photons, that increase measurement depth up to millimeters, and developing benchmarks for comparing the performance of commercial measurement systems.

At the end of the session, several important questions remained: (1) what is the maximum resolution and penetration depth of the optical methods, and (2) where should the measurement be performed (*e.g.*, in tissue, on a fluid-feed line supplying the tissue).

5.5. Mechanical Property Measurements

Discussion Leader: Dr. Callie Higgins (National Institute of Standards and Technology)

Traditional techniques for measuring the mechanical properties of materials, such as compression testing, tensile testing, atomic force microscopy, and nanoindentation, have significant limitations when used to measure biological tissues. For instance, they may be destructive, time-consuming, or unsuitable for very soft, highly deformable, small-volume materials. Alternatively, cross-correlative studies on cartilage tissue development using OCT, Raman spectroscopy, and optical coherence elastography can provide non-destructive and fast measurements of the spatial variation of mechanical properties. However, these methods are difficult to calibrate and are sensitive to environmental factors. Therefore, there is a need for developing new methods that can overcome these difficulties and measure mechanical properties accurately and reliably across different types of materials and applications.

Session participants were unsatisfied with state-of-the-art measurement techniques for biological samples. They claimed that the measurements are inaccurate, inconsistent and irrelevant. They emphasized the need for a reliable methodology to test mechanical properties in biological systems. They highlighted the challenges of analyzing highly deformable materials with internal structural variations. For example, nanoindentation measurements of hydrogels can yield widely different results between labs due to differences in how the measurements are conducted and the data are analyzed.

Participants raised several important questions that the community must address to increase reproducibility. For instance, what is the acceptable level of similarity to native tissue? Or what is the criterion for developing fully mature tissues (since the properties will change during the development)? They stressed the need for combinatorial technologies, and compositional, mechanical, and structural data. They noted that the relevant measurands for each TEMP will be application specific. However, they lamented the lack of a standard minimum threshold indicative of a clinical effect or successful product. They proposed to use existing data to generate those thresholds and values. They also expressed the need for better measurements at all steps of the development and manufacturing process. Finally, there is a need for better measurements of native tissues that can be used as design targets during research and development.

Finally, participants called for standards and best practices for cross-comparison of different technologies and approaches. One of the challenges in tissue engineering is a lack of reference materials that can mimic native tissue characteristics and serve as calibration standards for materials testing. For instance, silica is used as a reference for hard materials, but no such references exist for soft materials, like hydrogels. However, there is no consensus on what these soft material reference materials should be. Another challenge, particular to techniques that preserve tissue sterility (*e.g.*, acoustic elastography), is correlating measurements of

mechanical properties to the quality and efficacy of final product. Instrument developers should prioritize platforms that leverage miniaturization and integration of multimodal technologies for reliable, comprehensive, and non-destructive measurements of tissue samples. Participants stressed that a separate workshop on identifying measurements and standards for mechanical properties relevant for TEMPs would be beneficial to the field.

5.6. Sterility Measurements

Discussion Leader: Dr. Kirsten Parratt (National Institute of Standards and Technology)

Product sterility is critical for the successful commercialization of TEMPs. For example, regulatory agencies like the FDA require sterilization of medical devices in the final step of production [12]. Following validated sterility testing methods and standards during the production cycle can reduce the burden of the premarket review process.

Because product sterilization is a very specialized task, it is often outsourced from the main production facility. Traditional methods include incubation in peracetic acid or an ethanol solution, gamma or ultraviolet irradiation, and exposure to ethylene oxide gas [12]. When performing sterilization in-house (*e.g.*, steam, filtration), the users usually follow published methods from literature and aseptic techniques.

Sterilization is costly, laborious, and demands specialized skills and equipment. Labs typically sterilize after product finalization begins, using sterile environments initially to prevent contamination. However, sterile materials are more expensive. Controlling the lab environment involves managing supplies and safely disposing of high-risk biological materials, posing challenges for in-house implementation. Hence, there's an urgent demand for in-house sterilization methods with enhanced controls.

Sterilization for naturally derived materials (*e.g.*, decellularized extracellular matrix) is not straightforward and can affect the properties and function of the material being sterilized. For example, glutaraldehyde sterilization is common practice for commercially produced natural materials, such as collagen-based scaffolds, and is recognized for its capacity to alter the bioactivity and mechanical properties [13, 14]. Similarly, ultraviolet irradiation, for example, has been shown to cause bioactive modifications to the material [15].

Session participants agreed that to move the field of biofabrication forward, the community needs to develop sterilization methods that treat the full geometry of the material without affecting its properties. Nondestructive methods would be ideal but are not necessary for some materials. Improving in-house testing practices will raise confidence and minimize the likelihood of needing to repeat contamination tests. Finally, there is a need for a reference material that is completely sterile.

5.7. Measurements of Cell Distribution in Constructs

Discussion Leader: Taneka Jones (Vericel)

Most existing measurements for cell distribution within a construct rely on staining methods to label the cells. State-of-the-art techniques include confocal microscopy, wide-field fluorescence microscopy, light sheet microscopy, epifluorescence, and brightfield imaging. In cases where staining is not used, the microscopy data may suffer from low resolution. To get meaningful resolution, acquisition speed and throughput are reduced.

The limitation of most state-of-the-art techniques are throughput and the destructive nature of the measurement. Also, because microscopy inherently generates a large volume of data, the storage and analysis of this data remains challenging and costly. Small companies and start-ups struggle with adopting new technologies due to economic and infrastructure limitations. As such, session participants expressed that without standard reference materials or documentary standards, these companies rely on the hardware and informatic tools available to them, and on methods adapted from published literature.

A consistent and optimal cell concentration throughout the construct remains an outstanding challenge in the 3D printing of TEMPs. This can depend on several factors, such as: (1) whether the cells are adherent or not, (2) whether the cells are communicating with each other through signaling molecules, and (3) the end application of the printed TEMP. As such, it is important to monitor the behavior of cells throughout the 3D printing process. Currently, the cell concentration is determined by trial and error. A uniform loading unit (uniform delivery of cells) could ensure the cell density is accurate and reproducible for each print. The choice between culturing cells in 2D and 3D depends on the structural and functional requirements of the cells. Usually, 2D cell culture is preferred as a proof-of-concept because it is cost effective, rapid, and easier to image. However, 3D cell culture may be more relevant for applications that mimic the natural tissue environment.

The developers of manufacturing technologies are willing to sacrifice imaging resolution for higher throughput (in mm) of biomaterials and bioinks. They also need the technology to work with multiwell plates. A representative sample or construct should have a spatial scale of mm. The use of nanoparticles and magnetic particles could help scan the sample faster and track cells. However, different cell types may require individual reference materials to accurately measure their distribution within a construct; these standards should be application-specific to address the needs of the entire biomanufacturing community. A high-priority opportunity is to create a documentary standard that outlines the best practices for imaging different biomaterials and bioinks, including the experimental setup, the measurement technique, and the data analysis.

5.8. Measurements of Raw Materials

Discussion Leader: Rohan Shirwaiker (North Carolina State University)

In this context, raw materials for TEMPs include any and all synthetic and naturally derived materials used throughout the fabrication process. To reduce the burden of the regulatory process, it is often advantageous to use USP-grade raw materials which meet or exceed the requirements of the United States Pharmacopeia (USP). This is because research-grade materials require additional testing which can increase the cost and duration of the development process. However, USP-grade materials are not always meant for biologics. As such, there is an opportunity for the biofabrication community to identify high quality raw materials for development of TEMPs.

Existing measurement techniques for raw materials include ELISA (*e.g.*, for characterizing antibodies), NMR, swelling kinetics of polymer gels, and mass spectrometry fingerprinting. Often, these techniques are destructive and require a representative sample to be tested. This is particularly problematic for TEMPs that are expensive and challenging to manufacture. There is a lack of tools that support real-time measurements throughout the manufacturing process. These types of measurements could help identify sources of variability in the manufacturing pipeline and reduce production costs. Finally, the onboard data analytics on existing characterization instruments are poorly documented and lack transparency, leading many instrument users to write their own software.

In situations where raw materials from multiple suppliers are required to make the final product, material variability between manufacturers may become particularly problematic. Suppliers tend to use different techniques and metrics to purify and characterize their stocks, and these nuances may not be adequately documented on the specification sheets for each material. In fact, no reporting standards exist for product specification sheets. To minimize batch-to-batch variation, TEMP manufacturers conduct in-house tests on raw materials, such as rheological measurements, which are dependent on environmental conditions or equipment settings.

Session participants challenged suppliers of raw materials to publish information on how their materials are assessed and validated internally. At best, this type of information is sparse and varies widely in terms of quality metrics, preparation techniques, and characterization methodologies. Additionally, parsing the limited information from suppliers is costly for TEMP manufacturers and does not guarantee a reduction in product variability. Here, there is again the potential to develop reporting guidelines for specification sheets for raw materials. For example, in the case of a lyophilized bioink as a raw material, it is crucial to specify how the bioink is reconstituted (*e.g.*, in water or in cell culture media) for quality control measurements as this may differ from how TEMP manufacturers reconstitute the same bioink in their fabrication process. For cell sourcing, suppliers should report the sex, body mass index (BMI), and age of the tissue donor. These factors affect tissue manufacturing, as in the case of adipose-derived stem cells, which are sensitive to BMI. Currently, there are no community-accepted guidelines for the information cell suppliers should report. The community also needs

best practices and specifications for measuring protein concentration, cell viability and other bioactive markers.

5.9. Tissue Mimics

Discussion Leader: Lexi Garcia (The Advanced Regenerative Manufacturing Institute)

In this session, participants engaged in a robust discussion about the definition of tissue mimics and whether that definition is dependent on the manufacturing process or the function of the final product. Specifically, participants differentiated a 'functioning implant' used to replicate a biological function from a 'tissue' used to replace and integrate with native tissues. Participants agreed that a tissue mimic is more than a 3D construct and that it must replicate the functional, organizational, biochemical, and structural aspects of the native tissue both inside and outside the body.

Current approaches to mimicking different tissue structures includes the use of photopolymerization and 3D bioprinting. However, achieving appropriate resolution and batch-to-batch reproducibility remains challenging. Additionally, existing tissue mimics lack the complex 3D organization and functionality (*e.g.*, intercellular communication) of native tissues. This is in part because it is difficult to reproduce how ECM components (*e.g.,* RGD cell adhesion sites) are assembled. This is particularly important because the ECM stores growth factors which directly affect cell stability and response to the 3D environment; the ECM is responsible for tissue organization and maturation. Gas transport and vascularization in tissue mimics remain challenging as there are significant limitations in the resolution of the manufacturing process; specifically, sub-micrometer control has not been achieved with existing fabrication techniques. Scale-up also needs to be addressed as tissue mimics must scale to the size of native tissues (on the order of centimeters). Mimics must also be sectioned and assessed by histology to validate their structure. Participants also called for new technologies, beyond optical imaging, to characterize mimics, such as biosensors and antibodies for localized sampling.

5.10. Tissue Reference Data

Discussion Leader: Dr. Kaiming Ye (Binghamton University)

Participants engaged in a robust discussion about what constitutes tissue reference data and key considerations for how that data should be collected, organized, and stored. While further discussions are needed to set a clear definition and scope, participants did agree that some form of tissue reference data already exists (*e.g.*, ultimate tensile strength and maximum load for different tissues) throughout the methods and supplemental information in published literature [16, 17]. Unfortunately, this data can be hard to find, parse, and utilize in any meaningful way in part because it has been collected using methods that are not reproducible across different sites and operators. Moving forward, efforts are needed to collect, validate, and standardize tissue reference data, along with infrastructure to ensure comparability with

previously collected data. Ideally, tissue reference data should be stored in one place (*e.g.*, data portal), regularly maintained and updated, and freely available.

The participants voiced that two key considerations when collecting tissue reference data are (1) the preservation methods used to prepare tissues for testing and characterization and (2) the effects of those methods on the measurements themselves. For example, fixing a tissue with formaldehyde prior to mechanical testing may affect results [18]. As such, best practices or standard methods for tissue preparation and preservation could ensure comparability between different methodologies and improve the quality of tissue reference data.

Session participants agreed that while tissue reference data could be collected using destructive approaches, non-destructive methods would allow for validation studies of the same sample by multiple laboratories. To this point, participants recommended continued development of tools such as the atomic force microscopy (non-destructive), X-ray spectroscopy (high 3D resolution), OCT, ultrasound imaging, microwave light interference, magnetic resonance imaging (miniaturized), and broadband coherent anti-stokes Raman scattering (BCARS), with a focus on instrument miniaturization.

The session concluded with participants calling for more reference data on healthy tissues. This is because assessments of tissue structure, cellular composition, gene expression, and mechanical properties are often conducted on diseased tissues collected by surgeons in the operating theater.

6. Discussion

6.1. Types of Standards & Their Uses

When using this report to plan future work to improve measurements for the TEMPs field, it is important to delineate the common types of standards: reference materials, documentary standards and reference data [19].

- Documentary standards are classified into several types, but the most frequent ones are "guides" and "test methods." Guides are often a list important points to give advice on best practices for a measurement or process. Test methods focus on a specific measurement with a detailed protocol and should include information on repeatability (within the same lab: same operator and equipment) and reproducibility (between different labs, different operators, different equipment), usually requiring an interlaboratory study. Documentary standards should be developed by a consensus process, where all stakeholders that may be affected by the standard have an opportunity to provide input. The consensus process should include principles of fairness, openness, transparency, balance, and due process [19].
- Reference materials are physical artifacts that typically linked to a specific measurement to be used as a control when conducting the measurement, to establish that the measurement is giving the correct answer or to calibrate a measurement system.
- Reference data are carefully collected measurement data that can be used for comparison with experimental data to confirm that a measurement system is operating correctly, or to identify unknowns in a test sample. Reference data could be spectra or measurement values of properties of a material.

6.2. Documentary Standards

6.2.1. Considerations for Standard Test Methods

Workshop participants expressed frustration at the lack of measurement comparability for TEMPs and adamantly called for an array of standard test methods. For example, the concept of a standard test method for cell count was mentioned [20, 21]. While developing methods can be valuable, it doesn't imply that every cell counting method is suitable for everyone. There are instances where the standard test method may not be appropriate for a specific sample. Each cell preparation possesses unique properties that could render it incompatible with a particular counting method. Further, there is no authority to enforce the use of a standard test method: a researcher or product developer may use a method that is suitable for their product. Standard test methods are rigorously vetted, offering potentially higher reliability compared to unvetted methods. However, it's not a guarantee that they are the optimal choice or suitable for all scenarios.

6.2.2. Relevant Standards

SCB has developed a useful database (https://portal.standardscoordinatingbody.org/) that catalogs regenerative medicine standards. As of January 9, 2024, the database contains 364 standards from 29 organizations, 163 of which are relevant to TEMPs. This database is searchable and free to use. The following standards are particularly relevant to the discussion at the workshop:

- ISO 20391-1: Biotechnology - Cell counting - Part 1: General guidance on cell counting methods (2018)
- ISO 20391-2: Biotechnology - Cell counting - Part 2: Experimental design and statistical analysis to quantify counting method performance (2019)
- ASTM F2739 Standard Guide for Quantifying Cell Viability and Related Attributes within Biomaterial Scaffolds (2019)
- ASTM F3504 Standard Practice for Quantifying Cell Proliferation in 3D Scaffolds by a Nondestructive Method (2021) (describes the resazurin assay, also called Alamar Blue)
- ASTM F3510 Standard Guide for Characterizing Fiber-Based Constructs for Tissue-Engineered Medical Products (2021) (discusses test methods used for characterizing fiber-based scaffolds, such as electrospun scaffolds; tests include mechanical properties, fiber diameter and porosity)
- ASTM F3659 Standard Guide for Bioinks Used in Bioprinting
- ISO 10993-23 Biological evaluation of medical devices — Part 23: Tests for irritation (2021) (addresses use of a 3D reconstructed human epidermis for assessing skin irritation using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay)
- ISO 19007 - Nanotechnologies — In vitro MTS assay for measuring the cytotoxic effect of nanoparticles (2018) (has an interlaboratory study to assess precision for a nanotoxicity assay using cell cultures and the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide))
- ISO 23033 Biotechnology - Analytical methods - General requirements and considerations for the testing and characterization of cellular therapeutic products (2021)
- ISO 21560 - General requirements of tissue engineered medical products (2020)
- *New Standard under Development in ASTM F04:* Standard Test Method for Cumulative Population Doubling Analysis of the Proliferation of Vertebrate Tissue Cell Preparations (cell counting method for determining cumulative population doubling that includes interlaboratory study to assess precision)
- *New Standard under Development in ISO TC 276:* Biotechnology - General considerations and requirements for cell viability analytical methods - Part 1: Mammalian cells.

6.3. Reference Materials

The pre-workshop survey asked about the need for reference materials in tissue engineering. Figure 5 summarizes the responses. To design effective reference materials, we need to answer two questions: (1) what is the intended use of these materials? and (2) what measurement will they support? The most useful reference materials are tied to a particular measurement and can be used to assess measurement performance. For instance, the workshop participants suggested a “scaffold reference material”. This idea sounds simple, but it raises many questions. What kind of scaffold will it be? It could be made of electrospun fibers, porous ceramics, or 3D printed hydrogels. What measurement will it support? It could be used for mechanical or porosity tests. If it is for mechanical tests, what type of test will it be? Compression, tension, or nanoindentation? If it is for porosity tests, what method will it use? Gravimetry, porosimetry, or 3D imaging? If it is for 3D imaging, what technique will it use? Confocal fluorescence microscopy, OCT, or X-ray microcomputed tomography [22]? These questions are crucial to ensure that the reference material is fit-for-purpose and is appropriate for the measurand, the measurement technique, the application, and the community needs. Moreover, to justify the development of a reference material, we need to consider its usability and adoption by the tissue engineering community and whether standardization is worth pursuing.

One of the reference materials that was suggested in the survey (Fig. 5) was a synthetic material that mimics the properties of soft tissue for testing the strength of sutures: “Soft tissue analogs for tensile testing at the implant-tissue interface. Sawbones has bone analogs, yet we have little for soft tissue itself to compare our constructs to, or to assess fixation (with sutures or staples, etc.) as a replacement for cadaveric tissue testing”. This suggestion was notable as the proposer had considered how the reference material could be used in a specific measurement scenario.

6.4. Reference Data

The Foundation for Research on Information Technologies in Society (IT'IS) has developed a Tissue Material Properties Database (https://itis.swiss/virtual-population/tissue-properties/overview/) that is highly relevant to discussions at the workshop. The database aims to provide the “life sciences community with values for elemental composition, thermal, fluid, acoustic and magnetic resonance properties of biological tissues in a free, easily accessible, and dynamically evolving manner.” The database is drawn from the scientific literature and is continuously updated. The database includes information on 12 different tissue parameters: density, heat capacity, thermal conductivity, heat transfer rate, heat generation rate, dielectric properties, tissue frequency chart, low frequency (conductivity), viscosity, relaxation times, acoustic properties, and elemental composition. As an example, the density database contains values for 110 tissues including adrenal gland, blood, cortical bone, cancellous bone, and bone marrow. The average value, standard deviations, number of studies, minimum value, and maximum values are given for each entry. The database is quite an accomplishment and will be of great value to the TEMPs community.

7. Conclusion

This Report offers valuable input from key stakeholders in biomanufacturing and tissue engineering to guide future directions of TEMPs research and adoption for clinical applications. Major findings included the needs for non-invasive and non-destructive characterization methods and multi-parametric test methods that provide information on multiple product attributes in an integrated fashion. There is also a strong need for standards, such as standard test methods to assess product attributes, reference materials to assess measurement performance, and reference tissue data to provide reliable targets for tissue biofabrication. There is a consensus that lack of repeatability and reproducibility in common test methods used in industry (*e.g.*, cell viability assay, mechanical properties testing, and tissue functional assessment) is holding back adoption of TEMPs beyond the bench. It was felt that interdisciplinary collaborations in biomanufacturing would aid reproducibility and speed innovation. The future holds many exciting opportunities to improve measurements for TEMPs to enable improved performance and translation to clinical use.

Acknowledgements

The authors gratefully acknowledge the speakers, discussion leaders, and the workshop organizing committee for their time and energy in planning and hosting this event. In addition, we thank Dr. Katie Zander (technical program manager at the Standards Coordinating Body) for assistance with the pre- and post-workshop surveys, Laura Pierce and Eugenia Romantseva (NIST) for running the virtual meeting platform, taking detailed notes, and ensuring a great technical experience for all workshop participants.

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Appendix A. Supplementary Materials

A.1. Workshop Agenda

TECHNICAL PROGRAM		
Thursday, December 1, 2022		
10:00 AM	11:00 AM	Login to Bluejeans and Test System
11:00 AM	11:10 AM	Welcome Address: **Sheng Lin Gibson,** *NIST*
11:10 AM	11:45 AM	Plenary: Manufacturing complex biologics for regenerative medicine **Jennifer H. Elisseeff**, *Johns Hopkins University*
Session 1: Cell Viability **Discussion leader: Alicia Henn, *BioSpherix***		
11:55 AM	12:05 PM	Non-invasive non-contact real-time monitoring of cells within bio-reactors by direct imaging with optical coherence tomography **Naresh Menon**, *ChromoLogic*
12:05 PM	12:15 PM	Nondestructive Cell viability assessment using oxygen imaging **Mrignayani Kotecha**, *O2M Technologies*
12:15 PM	12:25 PM	Optical coherence tomography imaging for label-free measurement of cell viability in scaffolds **Carl Simon**, *NIST*
12:25 PM	12:55 PM	Panel Discussion
12:55 PM	1:25 PM	Lunch
Session 2: Cell Phenotype **Discussion leader: Jeff Halpern, *University of New Hampshire***		
1:25 PM	1:35 PM	Advancing Drug Discovery with Biofabricated 3D Tissue Models **Marc Ferrer**, *NCATS*
1:35 PM	1:45 PM	Protein Sensing **Marcie Black,** *Advanced Silicon Group*
1:45 PM	1:55 PM	Non-invasive quantitative live cell imaging **Kersti Alm**, *Phase Holographic Imaging*
1:55 PM	2:25 PM	Panel Discussion
2:25 PM	2:35 PM	Break
Session 3: Tissue Characterization **Discussion leader: Nathan Castro, *Nanochon Inc.***		
2:35 PM	2:45 PM	Dielectric spectroscopy for in line monitoring of engineered tissue constructs Rohan Shirwaiker, North Carolina State University
2:45 PM	2:55 PM	Title: TBD **Adam Feinberg**, *FluidForm*
2:55 PM	3:05 PM	Characterization of tissue engineered medical products

		Bao-Ngoc Nguyen, *FDA*
3:05 PM	3:35 PM	Panel Discussion
3:35 PM	3:45 PM	Break
3:45 PM	3:55 PM	Workshop Survey Review, **Carl Simon**, *NIST*
Breakout Session (Topics and Discussion Leaders) 1. Potency & phenotype measurements, **Richard McFarland**, *ARMI* 2. Cell viability measurements, **Mary Clare McCorry**, *ARMI* 3. Measurements of construct structure, **Kimberlee Potter**, *VA* and **Leanne Friedrich**, *NIST* 4. pH, O2 & metabolite measurements, **Zeeshan Ahmed**, *NIST* 5. Mechanical property measurements, **Callie Higgins**, *NIST* 6. Sterility measurements, **Kirsten Parratt**, *NIST* 7. Measurements of cell distribution in constructs, **Taneka Jones**, *Vericel* 8. Measurements of raw materials, **Rohan Shirwaiker**, *North Carolina State University* 9. Tissue mimics, **Lexi Garcia**, *ARMI* 10. Tissue reference data, **Kaiming Ye**, *Binghamton University*		
3:55 PM	4:35 PM	Breakout session
Closing Session		
4:35 PM	4:55 PM	Report back
4:55 PM	5:00 PM	Closing Remarks **Greta Babakhanova,** *NIST*

A.2. Workshop Participants: Biographies and Abstracts

Zeeshan Ahmed, Ph.D.
Chemist, Project Leader
National Institute of Standards and Technology
zeeshan.ahmed@nist.gov

Dr, Zeeshan Ahmed is currently a project leader in the Sensor Science Division at NIST where his research activities focus on the development of novel, disruptive technologies that aim to replace legacy-based measurement platforms. Specifically, his current research is focused on the development of physics constrained machine learning models for photonic and quantum sensors. This line of inquiry seeks to enable a cost-effective, integrated multi-functional sensor package with intelligent-calibration capabilities. He currently serves as Chairman of Task group on Emerging Technologies under Contact Thermometry at the Bureau International des Poids et Mesures (BIPM). In addition, he holds an Affiliate Faculty position with George Mason University's Chemistry department and the Quantum Science and Engineering Center (QSEC). His background includes work in data analytics, nanophotonics, Terahertz spectroscopy of biomaterials and pharmaceuticals, surface enhanced deep UV resonance Raman spectroscopy, and time-resolved vibrational spectroscopy of proteins. He received his PhD from University of Pittsburgh and BSc from George Mason University.

Kersti Alm, Ph.D.
Chef Scientific Officer
Phase Holographic Imaging (PHI)
kersti.alm@phiab.se

Dr. Alm has cooperated with PHI since 2005 and is a vital part of the PHI team since 2009. All insights gained through her PhD studies at Lund University, Sweden, her postdoc time at Roswell Park, Buffalo, NY, and the years after that working as a researcher and lecturer at Lund University, have been applied in the development of a genuinely novel cell analysis system. Her focus has been to develop a method that will neither cause cell stress nor harm while also providing accurate kinetic data. This goal has been reached both through in-house studies and collaborations as well as joint publications with researchers around the world, e.g., at Malmö and Lund universities, Northeastern University in Boston, UCSF in California, and many more.
Digital holographic imaging has existed as a brainchild since the '70s. Now, it is a reality since computers became powerful enough to handle the algorithms required to reconstruct the hologram of an imaged object – or even living cell cultures. PHI has proudly been part of this development and today advances cutting-edge research by applying digital holography to cell-based science.

Non-invasive quantitative live cell imaging

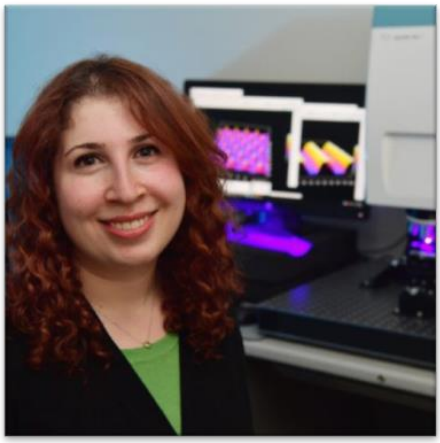
Abstract: Cells that will be used for clinical applications need to go through rigorous quality controls before being administered to patients. Many analyses require sacrificing parts of the precious sample and removing the sample from the production line. Label-free cell phenotype measurements can contribute to a wide scope of cell health and quality controls without losing any part of the sample and removing the sample from the product line. Digital holography is a label-free, robust, minimally destructive imaging method for cell analysis. The cells can stay in their preferred cell carriers in their preferred environment while being monitored continuously. The resulting quantitative data can be used for cell health controls, proliferation and viability measurements, differentiation monitoring, and even release criteria. My talk will briefly describe digital holography and how relevant cell phenotype data can be extracted.

Guillermo A. Ameer, Ph.D.
Daniel Hale Williams Professor
Northwestern University
g-ameer@northwestern.edu

Dr. Ameer is the Daniel Hale Williams professor of Biomedical Engineering and Surgery in the Biomedical Engineering Department at the McCormick School of Engineering and the Department of Surgery at the Feinberg School of Medicine, Northwestern University. He is the founding director of the Center for Advanced Regenerative Engineering (CARE). His research interests include regenerative engineering, biomaterials, additive manufacturing for biomedical devices, controlled drug delivery and bio/nanotechnology for therapeutics and diagnostics.

Dr. Ameer's laboratory pioneered the development and tissue regeneration applications of citrate-based biomaterials (CBB), the core technology behind the innovative bioresorbable orthopaedic tissue fixation devices CITREFIXTM, CITRESPLINETM, and CITRELOCKTM, which were recently cleared by the FDA for clinical use and marketed worldwide. CBBs are the first thermoset synthetic polymers used for implantable biodegradable medical devices. The co-founder of several companies, Dr. Ameer has over 300 publications and conference abstracts and 65 patents issued and pending in 9 countries.

His awards include the Key to the City of Panama, the Society for Biomaterials (SFB) Clemson Award for Contributions to the Literature, the SFB Technology Innovation and Development Award, the Chinese Association for Biomaterials Global Biomaterials Leadership Award, the Tissue Engineering and Regenerative Medicine Society Innovation and Commercialization Award, and the Bioactive Materials Lifetime Achievement Award. Dr. Ameer is a member of the National Academy of Medicine, and a Fellow of the American Institute of Medical and Biological Engineering (AIMBE), Fellow of the Biomedical Engineering Society, Fellow of the AIChE, Fellow of the American Association for the Advancement of Science (AAAS), Fellow of the Materials Research Society, and Fellow of the National Academy of Inventors. Dr. Ameer is a Deputy Editor for the AAAS journal Science Advances, Associate Editor for the Regenerative Engineering and Translational Medicine journal, a member of the board of directors of the Regenerative Engineering Society and AIMBE, and chair of the AIMBE College of Fellows.



Greta Babakhanova, Ph.D.
Physicist
National Institute of Standards and Technology
greta.babakhanova@nist.gov

Dr. Greta Babakhanova is a Physicist in the Biomaterials Group at the National Institute of Standards and Technology (NIST). She completed her Ph.D. in Chemical Physics from the Advanced Materials and Liquid Crystal Institute, Kent State University in 2019. She is a recipient of the 2020 Glenn H. Brown Prize awarded by The International Liquid Crystal Society for her outstanding contribution to the science of liquid crystals. After graduation, she was awarded the NIST-NRC Postdoctoral Research Fellowship (2019-2021) which enabled her to work on cell viability projects at NIST. Her current primary technical objective is to develop robust non-destructive methods for characterization of tissue engineered medical products. Since April 2022 Dr. Babakhanova is also working in the Program Operations Division at the Office of Advanced Manufacturing, where she assists with the program management of NIST-funded initiatives.

Marcie Black, Ph.D.
Co-founder & CEO,
Advanced Silicon Group
marcie@advancedsilicongroup.com

Dr. Marcie Black is the co-inventor of the silicon nanowire array biosensor. Dr. Black brings expertise in building strong teams, managing development projects, patents, IP strategy, encouraging a healthy company culture, cost modeling, and running a startup. Prior to founding ASG, Marcie was the co-founder of Bandgap Engineering, which focused on lowering the cost of solar electricity through black silicon or silicon nanowire solar cells. Bandgap raised over $10 million of investment from VCs, angels, state, and national grants. In 2009, she was awarded an R&D 100 award for her contributions to work at LANL. Marcie also was honored as one of the ten "Women-to-Watch in 2010" by Mass High Tech.

Protein Sensing

Abstract: Dr. Black will discuss basic methods for measuring cell phenotype – especially proteins – and review some of the advantages and challenges for these. Then she will present the photoelectric ELISA biosensor, LightSense. LightSense will be able to detect a wide range of protein concentrations, monitor multiple proteins simultaneously, and measure proteins at low-concentrations even when submerged in a high-concentration of another protein and do so at a low-cost. Lastly, Dr. Black will point out a few other technologies that she finds promising to meet the market need for sensing.

Billyde Brown, Ph.D.
Senior Research Faculty, EWD Director
Georgia Tech Manufacturing Institute
billyde.brown@gatech.edu

Dr. Billyde Brown is a Senior Research Faculty and Education and Workforce Development (EWD) Director at the Georgia Tech Manufacturing Institute (GTMI), one of eleven interdisciplinary research institutes at the Georgia Institute of Technology. Dr. Brown's overall role is to create strong partnerships among industry, government, and academia in the area of manufacturing research, development, and deployment, while acquiring and managing sponsored research programs.

Dr. Brown is currently the PI of an Advanced Regenerative Manufacturing Institute (ARMI BioFabUSA) funded project to develop a wireless sensor system for real-time in-situ monitoring of critical quality attributes including pH, glucose, lactate, and protein biomarkers in human mesenchymal stem cell expansion bioreactors. Dr. Brown manages a Manufacturing Certificate program and proctors a Manufacturing Seminar Course for the Georgia Tech College of Engineering while hosting a 10-week GTMI Lunch and Learn Lecture Series each semester with high profile industry, government, and academic speakers to share advanced manufacturing knowledge within a global community. Dr. Brown also coordinates an annual 10-week Research Experience for Undergraduates (REU) summer program called REVAMP (Research Experiences for student Veterans in Advanced Manufacturing and entrePreneurship) sponsored by the National Science Foundation that maintains target demographics of 50% student veterans and 40% underrepresented minorities with STEM majors. Dr. Brown has strong expertise in several technical areas including electrochemical biosensors, nanomaterial synthesis and characterization, thin-film additive manufacturing, and electrochemical energy storage. Dr. Brown has over 20 peer-reviewed publications and is a regular reviewer of high impact factor peer reviewed journals. He earned his B.S. and Ph.D. degrees in Electrical Engineering from NC State University and Duke University, respectively.

Nathan J. Castro, Ph.D.
Co-founder
Nanochon, Inc. Inc.
nathan.castro@nanochon.com

Dr. Nathan J. Castro is an experienced biomedical researcher and entrepreneur. He has assisted in the design, testing, regulatory approval and clinical translation of 3D printed medical devices, as well as co-founded Nanochon, Inc. He is skilled in policy analysis, life sciences, data analysis, and quantitative research with particular interest in clinical translational (bench-to-bedside).

Jennifer H. Elisseeff, Ph.D.
Morton Goldberg Professor
Director of the Translational Tissue Engineering Center
Johns Hopkins University
jhe@jhu.edu

Dr. Elisseeff is the Morton Goldberg Professor and Director of the Translational Tissue Engineering Center at Johns Hopkins Department of Biomedical Engineering and the Wilmer Eye Institute with appointments in Chemical and Biological Engineering, Materials Science and Orthopedic Surgery. She was elected a Fellow of the American Institute of Medical and Biological Engineering, the National Academy of Inventors, a Young Global Leader by World Economic Forum. In 2018, she was elected to the National Academy of Engineering and National Academy of Medicine and in 2019 she received the NIH Directors Pioneer Award. In 2022, she was elected a fellow of the American Association of Arts and Sciences.

Jennifer received a bachelor's degree in chemistry from Carnegie Mellon University and a PhD in Medical Engineering from the Harvard–MIT Division of Health Sciences and Technology. Later she was a Fellow at the National Institute of General Medical Sciences, Pharmacology Research Associate Program, where she worked in the National Institute of Dental and Craniofacial Research. She is committed to the translation of regenerative biomaterials and has founded several companies and participates in several industry advisory boards including appointment by the governor to the State of Maryland's Technology Development Corporation. Dr. Elisseeff's laboratory has developed technologies licensed to startups, small and large companies. Lab-grown products have reached clinical testing as drugs, biologics, and devices in orthopedics, plastic and reconstructive surgery and lab is now running a Phase 2 clinical trial. She has served on an FDA panel and presented products to the FDA.

Jennifer's initial research efforts focused on the development of biomaterials for studying stem cells and designing regenerative medicine technologies for application in orthopedics, plastic and reconstructive surgery, and ophthalmology. Clinical results revealed the importance of the immune response in the biomaterial and regenerative medicine responses. This led to a significant shift in research efforts to biomaterials-directed regenerative immunology and leveraging the adaptive immune system to promote tissue repair. The group is now characterizing the immune and stromal environments of healing versus non-healing wounds and tumors. Biomaterials are now being applied to model and manipulate tissue environments and studying the impact of systemic and environmental factors such as aging and senescent cells, sex differences, and infection/microbiome changes on tissue homeostasis and repair. She has extensively published and lectured nationally and internationally on these topics.

Manufacturing complex biologics for regenerative medicine

Abstract: Tissue repair is a coordinated process that includes multiple immune and stromal cells working in concert to remove damage and rebuild matrix. Therapies that target multiple cell types and pathways in the repair process, and combination therapies, will likely be most effective in rebuilding tissue. Complex products such as biological scaffolds derived from the extracellular matrix (ECM) of tissues contain many components that can more broadly target the network of cells that promote wound healing. New regenerative immunotherapies derived from helminth soluble egg antigens contain a multitude of proteins and lipids that together stimulate tissue repair. Without a single agent mechanism of action, these complex products can be difficult to characterize with
respect to variability in batch-to-batch composition and biological activity. This presentation will discuss regenerative immunotherapies with complex composition and methods for screening efficacy and product development.

Adam Feinberg, Ph.D.
Co-Founder and Chief Technology Officer
FluidForm Inc,
adam@fluidform3d.com

Dr. Adam Feinberg is Co-Founder and CTO of FluidForm Inc, a start-up company commercializing FRESH 3D bioprinting technology, and a Professor at Carnegie Mellon University in the departments of Biomedical Engineering and Materials Science & Engineering. He earned his BS in Materials Science & Engineering from Cornell University in 1999 with Co-op experience at Abiomed, Inc., working on total artificial hearts. He then earned his PhD in Biomedical Engineering from the University of Florida, focused on engineering cell-material interactions to prevent and enhance adhesion. This was followed by postdoctoral training at Harvard University, developing new biomaterials and stem cell-based cardiac tissue engineering strategies.
Dr. Feinberg has co-authored over 50 peer-reviewed publications, holds over 20 US patents and patent applications, and has received multiple honors including the NSF CAREER Award, the NIH Director's New Innovator Award, and Fellow of the American Institute for Medical and Biological Engineering. A primary research focus is engineering extracellular matrix (ECM) protein scaffolds using advanced biomanufacturing and 3D bioprinting approaches for multiple applications including cancer models, regenerative scaffolds, skeletal muscle, and cardiac muscle tissue engineering. At FluidForm, he is driving the commercialization of the FRESH 3D bioprinting platform for a wide range of applications in the pharma, medical device, and regenerative medicine industries.

In situ volumetric imaging and analysis of 3D bioprinted constructs using optical coherence tomography

Abstract: As 3D bioprinting has grown as a fabrication technology, so too has the need for improved analytical methods to characterize engineered constructs. This is especially challenging for engineered tissues composed of hydrogels and cells, as these materials readily deform when trying to assess print fidelity and other properties non-destructively. Establishing that the 3D architecture of the bioprinted construct matches its intended anatomic design is critical given the importance of structure-function relationships in most tissue types. I will present the development of a multimaterial bioprinting platform with integrated optical coherence tomography for in situ volumetric imaging, error detection, and 3D reconstruction. This enables quantitative 3D volumetric imaging with micron resolution over centimeter length scales, the ability to detect a range of print defect types within a 3D volume, and real-time imaging of the printing process at each print layer. These advances provide a comprehensive methodology for print quality assessment, paving the way toward the production and process control required for achieving regulatory approval and ultimately clinical translation of engineered tissues.

Marc Ferrer, Ph.D.
Director, 3-D Tissue Bioprinting Laboratory
National Center for Advancing Translational Sciences
marc.ferrer@nih.gov

Dr. Ferrer is the Director of the National Center for Advancing Translational Sciences (NCATS) 3D Tissue Bioprinting Laboratory, a multidisciplinary group with the goal of creating, validating, and using 3D bioengineered tissues for disease modeling and drug discovery and development. Previously, Marc was a Team Lead at the NIH Chemical Genomics Center working on the discovery of small molecule probes to study protein function. Before joining NIH, he was Director of Assay Development and High Throughput Screening at the Department of Automated Biotechnology at the Merck Research Laboratories. Marc received a BSc degree in Organic Chemistry from the University of Barcelona, and a Ph.D. degree in Biological Chemistry from the University of Minnesota.

Advancing Drug Discovery with Biofabricated 3D Tissue Models

Abstract: The NCATS 3D Tissue Bioprinting Laboratory (3DTBL) is a multidisciplinary laboratory with experts in bioengineering, assay development, HTS, automated biology and cell microscopy with the goal of creating a versatile and robust platform of biofabricated 3D tissue models for drug screening. We are assembling a portfolio of biofabricated normal and disease 3D tissue models that recapitulate faithfully the morphology, physiology and pathology of human tissues. These biofabricated 3D tissue models are produced in a multiwell-plate format with disease relevant phenotypic assays to quantitatively assess drug efficacy and toxicity. The expectation is that these physiologically relevant 3D tissue models will be clinically predictive cellular assays for drug discovery and development.

Leanne Friedrich, Ph.D.
Material Scientist
National Institute of Standards and Technology
leanne.friedrich@nist.gov

Dr. Leanne Friedrich (she/her) is a materials scientist at the National Institute of Standards and Technology working on embedded 3D printing of soft materials. Leanne earned a B.S. in materials science and engineering from Northwestern University in 2015. She earned a Ph.D. in materials from University of California Santa Barbara in 2020, developing methods to 3D print polymer matrix composites with controlled property gradients using direct ink writing with acoustophoresis. She joined NIST in 2020 as an NRC postdoc. Leanne's work focuses on developing guidelines for material selection and printing parameter selection in embedded 3D printing. By examining how the rheology of the ink, rheology of the support, and interfacial tension influence defects in printed structures, we can achieve more reliable prints with better shape fidelity, mechanical integrity, and functional properties. Leanne uses computational fluid dynamics simulations in OpenFOAM to study the underlying physics of the printing process, and she uses in-situ imaging experiments to measure defects in printed structures.

Lexi Garcia, M.S.
Director of Strategic Projects
Advanced Regenerative Manufacturing Institute (ARMI)
lgarcia@armiusa.org

Lexi Garcia is the Director of Strategic Projects at the Advanced Regenerative Manufacturing Institute (ARMI) | BioFabUSA. As such she is responsible for coordinating and leading cross-functional teams to drive key project activities to help shape the long-term vision of the company. She leads ARMI | BioFabUSA's strategic standards initiatives, which includes guiding both ARMI members and other tissue engineered medical product (TEMP) subject matter experts through the standards development process, particularly those related to bioprinting. Lexi has a wide TEMP network and works to facilitate connections between key stakeholders throughout the tissue engineering community to enable partnerships that will propel the field forward. With degrees in Neuroscience from Middlebury College, and Conservation Biology and Sustainable Development from the University of Maryland, College Park (UMD) she has a unique systems perspective and approach to nurturing the tissue engineering ecosystem to garner consensus and improve collaborative efforts.

Jeffrey Halpern, Ph.D.
Associate Professor
University of New Hampshire
Jeffrey.Halpern@unh.edu

Prof. Jeffrey M. Halpern (he/him) earned his PhD in Chemical Engineering in 2010 at Case Western Reserve University in Chemical Engineering. Halpern pursued a first postdoc (2011-2012) funded through an NIH NRSA fellowship at Case Western Reserve University in Biomedical Engineering and a second postdoc through Fulbright and Lady Davis Fellowships (2013-2014) in Israel at the Technion in the Department of Chemical Engineering. He joined the Department of Chemical Engineering at the University of New Hampshire. Recently, Halpern leads an NSF EPSCoR Track II (#2119237) team the development of "universal" biotechnology sensing platforms to enable the use of advanced manufacturing principles in biomanufacturing. As a collaboration across four states, NH, AL, ME, and WY, we aim to develop engineering principles to guide on-demand biosensor design covering the full assortment of sensor components: the recognition elements that bind analytes; the stimuli-responsive linkers that amplify the binding signal; the form factor of the sensor; and the final transduction of the signal. You can read more about our team here.

Dawn Henke, Ph.D.
Senior Technical Program Manager
Standards Coordinating Body (SCB)
dhenke@regenmedscb.org

Dr. Dawn Henke has extensive experience in the advanced biological sciences and has played an active role in the regenerative medicine community throughout her career. Prior to joining SCB, she worked as a post-doctoral fellow at the National Institutes of Health, National Eye Institute performing stem cell research focused on developing retinal organoids from stem cells for testing and therapeutic purposes. She holds a PhD in Genetics and Genomic Sciences from the University of Alabama at Birmingham. As Senior Technical Program Manager, Dawn is the supervisor of the program manager team and acts as a liaison between the program managers and the executive director.

Alicia Henn, Ph.D., MBA
Chief Scientific Officer
BioSpherix, Ltd.
ahenn@biospherix.com

Alicia D. Henn PhD MBA is Chief Scientific Officer for BioSpherix, Ltd. Previously, Dr. Henn was an academic researcher in the cancer and immunology fields. Dr. Henn owns the In Vitro Reproducibility and Physiologic Cell Manufacturing groups on LinkedIn, promoting clonable, physiologically relevant cell environments for better scientific reproducibility and translatability.

Callie Higgins, Ph.D.
Materials Engineer, Co-Project Leader
National Institute of Standards and Technology
callie.higgins@nist.gov

Dr. Callie Higgins is the Co-Project Leader of the Photopolymer Additive Manufacturing (PAM) Project at the National Institute of Standards and Technology (NIST) in Boulder, CO and is an adjunct faculty at the Colorado School of Mines. Her work with Co-Project Leader, Jason Killgore, studying the fundamental properties of PAM systems was recently the sole awardee of one of the Federal Government's highest honors, the Samuel J. Heyman Service to America Medal (SAMMIES) for Emerging Leaders. She graduated with her PhD from CU Boulder's Department of Electrical Engineering with specialties in optics and material science where she characterized the fundamental properties of photopatterned hydrogels for use in regenerative medicine. Outside of the lab, she loves to adventure around the mountains; skiing, hiking, and picnicking the whole way up with her husband, friends, and family.

James (Jay) Hoying, Ph.D.
Partner and Chief Scientist
Advanced Solutions Life Sciences
jhoying@advancedsolutions.com

Dr. James (Jay) Hoying is a Partner and Chief Scientist at Advanced Solutions Life Sciences with more than 30 years of experience in basic and applied biological sciences with a focus in tissue biology, tissue vascularization, and tissue fabrication. Prior to joining ASLS, he was Professor and Chief of the Division of Cardiovascular Therapeutics at the Cardiovascular Innovation Institute (CII) where he developed a broad background in tissue fabrication, cell therapeutics, and translation of discoveries to industry and the clinic. Dr. Hoying is an inventor of the AngiomicsTM microvessel technology and holds numerous patents related to vascularizing tissues and related cell-based therapies. Dr. Hoying received his BA and MS degrees in Biology and Molecular Biology from Case Western Reserve University and his PhD in Cardiovascular Physiology, with an emphasis on the microcirculation, from the University of Arizona. Following degree work, Dr. Hoying served as a New Investigator in the National Institutes of Health Program of Excellence in Molecular Biology of the Heart and Lung (POEMB). He currently serves on the Editorial staff of national scientific journals and reviews for individual and program grant proposals for the National Institutes of Health, the Veterans Affairs, the American Heart Association, and international funding agencies. Dr. Hoying is also a Fellow of the American Heart Association.

John Huang, Ph.D.
Founder & Chief Executive Officer
TheWell Bioscience, Inc
johnh@thewellbio.com

Dr. John Huang is an inventor and entrepreneur in advanced biomedicine and bioengineering with a number of patented technologies. He is actively involved in product development and commercialization with great enthusiasm for novel biotechnologies and their influences on the life science industry. John is the founder and CEO of TheWell Bioscience, Inc., a pioneer in the 3D biomimicking platform for precision medicine, cell therapy, and biomanufacturing. The company uses its state-of-art biomatrix system to closely mimic the natural extracellular matrix of the human microenvironment and establish a robust 3D cell culture platform and smart delivery system to advance drug discovery, tissue engineering, cell therapy, and personalized medicine. The company received multiple awards, including Most Innovative 3D Hydrogel System in 2020 Healthcare & Pharmaceutical Awards, Listed as #1 of 8 Top 3D Bioprinting Startups Impacting The Biotechnology Industry, and Leading Innovators in Cell Therapy Research 2021 Healthcare & Pharmaceutical Awards. John served as an editorial board member and ad-hoc reviewer of 11 academic journals. He is the recipient of the Anheuser-Bush fellowship, the research foundation award, and 22 new ingenuity awards.

Taneka Jones, Ph.D.
Medical Science Liaison
Vericel Corporation
tjones@nsbe.org

Dr. Taneka Jones is a visionary and inventor. Inspired by her ancestors, education is highly valued. She received a Ph.D. and a Master of Science Degree in Bioengineering from the University of Illinois-Chicago. Additionally, she holds a M.A in Teaching and earned a B.S. in Biological Sciences with a minor in Chemistry.

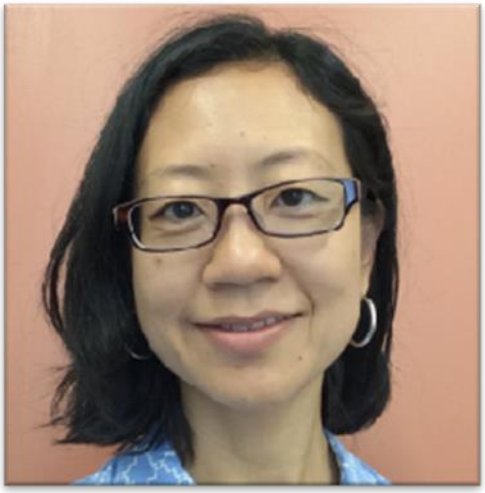
Dr. Jones served as a licensed professional educator with Chicago Public Schools while completing her graduate studies, and credits her continued success to her faith, which is her guidepost. In her current role as a Medical Science Liaison, she interfaces between academia and industry for an FDA-approved cell therapy product. A rising entrepreneur, her research consulting company Biopraise seeks to educate, motivate, and empower students, educators, administrators, and biotechnology stakeholders. She has designed and implemented three entry-level programs in the science, technology, engineering, and mathematics (STEM) fields to enhance and empower youth in disadvantaged areas to reach this goal. Her five favorite hobbies include international travel, outdoor adventures, middle- and long-distance running, watching documentaries and enjoying a great cup of coffee.

Mrignayani Kotecha, Ph.D.
President
O2M Technologies, LLC
mk@oxygenimaging.com

Dr. Mrignayani Kotecha is a founding member and the president of Chicago-based biotech company O2M Technologies, LLC. In this role, Dr. Kotecha provides guidance and leadership for developing the world's first EPR oxygen imaging preclinical and clinical instruments, accessories, synthesis of oxygen-sensitive spin-probes, scientific research, fundraising, and outreach. She leads the "Oxygen Measurement Core" research facility at O2M that performs cutting-edge research involving oxygen imaging in cancer and regenerative medicine. Dr. Kotecha holds a Ph.D. in Physics from Jabalpur University, India and has over 25 years of experience in magnetic resonance technologies. She has published over fifty peer-reviewed articles and is the lead editor of the 2017 Wiley book "Magnetic Resonance Imaging in Tissue Engineering".

Nondestructive Cell Viability Assessment Using Oxygen Imaging

Abstract: Cell viability is an essential parameter for cell therapy, tissue engineering, drug screening, and many other biological processes and products. These products rely on viable, healthy, and functional cells to work as intended for solving various medical conditions, such as cancer, type I diabetes, arthritis, liver, kidney, bone damage, neurodegenerative, cardiovascular damage, etc. However, current methods that rely on assays to measure cell viability are destructive and inadequate for three-dimensional tissues. Besides, these methods do not assess cell functionality, a key parameter for cell therapy and tissue engineering medical products. In addition, these methods have not been tested for their interference with biomaterials commonly used in the field, therefore, may provide an inaccurate assessment when used with artificial tissue grafts involving scaffolds. Electron paramagnetic resonance oxygen imaging (EPROI) is a noninvasive oxygen mapping method with high precision and absolute accuracy. Similar to nuclear magnetic resonance imaging (MRI), EPRI uses magnetic field gradients to generate the spatial distribution of electron spins. In contrast to conventional MRI, EPRI relies on a much smaller magnetic field (in the milli Tesla range), generated by cryogen-free magnets and gradients that do not change during signal detection. EPROI uses the linear relationship between electron spin-lattice relaxation rate and partial oxygen pressure (pO2) of an injectable non-toxic soluble contrast agent, trityl OX071, for obtaining oxygen maps in tissues. We have developed the world's first dedicated 25 mT preclinical EPROI instrument JIVA-25™, which operates at 720 MHz radiofrequency.
I will present the concept and data showing how EPROI can be used for noninvasive cell viability assessment and why it is superior to current methods of cell viability measurements. This is the first demonstration of noninvasive cell viability assessment in a three-dimensional system without destroying the cells or scaffold. The method can be extended to perform cell viability and functionality assessment for all tissue engineering medical products of any size and dimensions.



Sheng Lin-Gibson, Ph.D.
Chief of the Biosystems and Biomaterials Division
National Institute of Standards and Technology (NIST)
sheng.lin-gibson@nist.gov

Dr. Sheng Lin-Gibson is the Chief of the NIST Biosystems and Biomaterials Division. She oversees a multidisciplinary research portfolio that includes regenerative medicine and advanced therapies, precision medicine, synthetic biology, and complex microbial systems. She leads and coordinates the development of global standards for emerging biotechnology, many of which support regenerative medicine and advanced therapy. She has coauthored over 80 peer-reviewed publications, serves on many Interagency Working Groups as well as numerous expert review panels and advisory boards. She has received two Department of Commerce Gold Medals.

Mary Clare McCorry, Ph.D.
Director for Technology and Process Development
Advanced Regenerative Manufacturing Institute
mmccorry@armiusa.org

Dr. Mary Clare McCorry is the Director for Technology and Process Development at the Advanced Regenerative Manufacturing Institute (ARMI). At ARMI, Dr. McCorry is advising the development of several scalable, modular, automated and closed tissue manufacturing production lines as well as leading the development of novel manufacturing technologies. As part of her role, Dr. McCorry is responsible for directing Institute funded technical projects and strategic partnerships to advance manufacturing technologies in cell, tissue, and organ engineering. Prior to joining ARMI, she was an American Institute for Medical and Biological Engineering (AIMBE) Scholar at the U.S. Food and Drug Administration in the Center for Devices and Radiological Health (CDRH). As an AIMBE Scholar, she led science policy initiatives and coordinated collaborations between experts in academia, industry, government and non-profit organizations. Mary Clare joined FDA from Cornell University where she designed cell-based assays to study biomechanical/chemical mechanisms of action of cells in tissue engineered constructs. She also consulted for industry on the design of specialized single use tissue bioreactors. Mary Clare received her Ph.D. from Cornell University and her BS in Biomedical Engineering from Worcester Polytechnic Institute.

Richard McFarland, Ph.D., MD
Chief Regulatory Officer, Advanced Regenerative Manufacturing Institute
Principal Consultant, BioFabConsulting
rmcfarland@armiusa.org

Richard McFarland is an immunopathologist and the Chief Regulatory Officer at the Advanced Regenerative Manufacturing Institute (ARMI) where he over the regulatory affairs for ARMI and its BioFabUSA program. Dr. McFarland is also a Principal Consultant at BioFabConsulting where he consults with members on product classification, regulatory strategy, and CMC, preclinical and clinical studies. Prior to joining ARMI as its first post-award hire in 2017, Dr. McFarland was Associate Director for Policy (ADP) of the Office of Tissues and Advanced Therapies (and its predecessor office) at the Food and Drug Administration's Center for Biologics Evaluation and Research (FDA/CBER) for eleven years after six years a reviewer in FDA/CBER. In addition he, served on the federal government's interagency for tissue engineering and regenerative medicine, the Multi-agency Tissue Engineering Sciences group (MATES) for fifteen years, including five years as its Chair. Dr. McFarland received his undergraduate, graduate, and medical school training at the University of North Carolina-Chapel and his post-graduate medical specialty training in anatomic/clinical pathology and subspeciality training in immunopathology at University of Texas Southwestern Medical Center in Dallas.

Naresh Menon, Ph.D.
Chief Executive Officer
ChromoLogic
nmenon@chromologic.com

As the founder of ChromoLogic, Dr. Menon is passionate about developing novel biomedical solutions that not only lead to superior patient outcomes but also ensure that the solutions are cost effective and affordable. He received his PhD in Physics from Purdue University with an emphasis in sensor fabrication, instrumentation and novel data analytic methods that were applied at multiple national and international laboratories towards fundamental physics discoveries. His early career was spent at Corning Incorporated and Northrop Grumman Mission Systems where he was groomed for leadership positions in multiple businesses.

Founded in 2007, ChromoLogic is a boutique product focused innovation center developing solutions that save lives and make the world secure. The Biomedical Solutions division develops point of care solutions with the goal of delivering best health outcomes at the lowest cost. Their capabilities span four key verticals: Wound Care and Infectious Disease, Diagnostics/Screening, Drug Delivery, and Telehealth. Their customers and collaborators include medical manufacturers, academic institutions, government agencies, and care providers. To meet the needs of this broad customer and market base, they bring together the brightest minds from every engineering and science discipline and form collaborations across academia and industry. Since 2019, ChromoLogic has made significant investments in cell therapy, in terms of therapeutics as well as instrumentation. Their current programs include a novel genetically modified bacteria that delivers cytokines to manage side effects from cancer treatment and an optical system that can non-invasively monitor cell growth, concentration & viability within bioreactors in real-time.

Non-invasive non-contact real-time monitoring of cells within bio-reactors by direct imaging with optical coherence tomography

Abstract: We have developed OCTiCell for monitoring cell growth in suspended agitated bioreactors based on optical coherence tomography. OCTiCell is an in-line, completely non-invasive instrument that can operate on any suspended-cell bioreactor with a window or transparent wall. In traditional optical coherence tomography, the imaging beam is rastered over the sample to form a three-dimensional image. OCTiCell, instead uses a fixed imaging beam and takes advantage of the motion of the media to move the cells across the interrogating optical beam.

Bao-Ngoc Nguyen, Ph.D.
Biomedical Engineer
The Food and Drug Administration
Bao-Ngoc.Nguyen@fda.hhs.gov

Dr. Bao-Ngoc Nguyen is a Biomedical Engineer in the Tissue Engineering Branch (TEB) of the Division of Cellular and Gene Therapies (DCGT) in the Office of Tissues and Advanced Therapies (OTAT)/Center for Biologics Evaluation and Research (CBER). She conducts regulatory review of cellular therapies, tissue engineered products, and devices regulated in OTAT, focusing on the chemistry, manufacturing, and controls of these products. Dr. Nguyen earned her B.S. and Ph.D. in Bioengineering at the University of Maryland, College Park.

Characterization of Tissue Engineered Medical Products

Abstract: Tissue engineered medical products (TEMPs) are complex and require assessment of the cells, scaffold, and final cell-scaffold product. Depending on the TEMP, final product release testing may require use of parts or entire final products. When only parts of the final product or surrogate products are used, sufficient supporting data may be necessary to demonstrate that they adequately represent the clinical product. Early consideration of final product release testing methods is critical in supporting the safety and effectiveness of TEMPs.

Kirsten Parratt , Ph.D.
Biomedical Engineer
National Institute of Standards and Technology
kirsten.parratt@nist.gov

Dr. Kirsten Parratt is a Biomedical Engineer in the Biosystems and Biomaterials Division at the National Institute of Standards and Technology (NIST). Currently, she develops flow cytometry experimental and computational methods to characterize live, whole cell, microbial materials. This work supports biomanufacturing stakeholders, for example, those interested in Live Biotherapeutic Products and Rapid Microbial Testing Methods for advanced therapy products. Kirsten obtained her BSE in Chemical Engineering from Princeton University. She obtained her MS in Materials Science and Engineering, and PhD in Bioengineering from the Georgia Institute of Technology, where her graduate dissertation was related to stem cell biomanufacturing and tissue engineering.

Marian S. Piekarczyk, M.S., MBA
Senior Regenerative Medicine Technical Specialist
Thermo Fisher Scientific
marian.piekarczyk@thermofisher.com

Marian S. Piekarczyk is a Senior Regenerative Medicine Technical Specialist with Thermo Fisher Scientific within their Life Sciences Division. Her background is in stem cell science and biology and immunology. Marian currently leads business development for cell therapy and translational medicine applications in the central Midwest region of the United States. Her career spans over 30 years in academic research, small biotech, and industry related research, technical, and commercial operations.

Laura Pierce, M.S.
Biomedical Engineer
National Institute of Standards and Technology
laura.pierce@nist.gov

Laura Pierce is a Biomedical Engineer in the Biomaterials Group at the National Institute of Standards and Technology. She is responsible for technical planning and conducting studies to support measurement capabilities and assurance for cell therapy products. In that capacity, she routinely performs cell counting measurements on a variety of lab instrumentation typically used in the Regenerative Medicine field, and she supports analysis and dissection of data with collaborators to define the points in their experimental workflow which introduce measurement error and uncertainty and offers recommendations to improve precision and robustness of measurements. Laura has supported the development of the Cell Counting Part II ISO standard and COMET application for comparison of cell counting methods, and is currently involved in the development of a draft Cell Viability consensus standard through the International Standards Organization.

Kimberlee Potter, Ph.D.
Scientific Program Manager
Department of Veterans Affairs
Kimberlee.Potter@va.gov

Dr. Potter joined the Office of Research and Development at the Department of Veterans Affairs in 2011, where she is the Scientific Portfolio Manager of Surgery, Trauma, and Restorative Medicine. She currently serves as the VA representative on the Forum for Regenerative Medicine at the National Academies of Sciences, Engineering, and Medicine, the Armed Forces Institute of Regenerative Medicine Oversight Committee, and CDMRP's Programmatic panel for the Reconstructive Transplantation Research Program. She received her undergraduate degree in Engineering Chemistry from Queen's University in Canada and her Ph.D. from Cambridge University in England. She joined the National Institutes of Health as a visiting scientist and she spent 10 years at the Armed Forces Institute of Pathology as the Technical Director of the Magnetic Resonance Microscopy Facility where she applied non-invasive imaging techniques to the study of forensic, pathologic, and engineered tissues. Dr. Potter credits her training in Chemical Engineering, Chemistry, Medical Imaging, and Tissue Engineering as the ideal framework to support the translation of biofabrication solutions to the operating room.

Itedale Namro Redwan, Ph.D.
Chief Scientific Officer
CELLINK
inr@cellink.com

Dr. Namro Redwan holds a PhD degree in Chemistry with emphasis on Medicinal/Organic Chemistry from the University of Gothenburg. She obtained her Postdoctoral training in Chemical Biology at the Gladstone Institutes in San Francisco. She was an Assistant Professor at the Clinical Chemistry and Transfusion Medicine at the University of Gotehnburg prior to joining CELLINK as a Senior Principal Scientist/Scientific Officer. Today, Itedale is the Chief Scientific Officer, currently leading the Science and Applications team in the R&D department at CELLINK.
CELLINK is the world leading Bioprinting company and Itedale's team has commercialized 45+ bioinks and published 15+ application notes in the field of Biofabrication.

Jane Romantseva, M.S.
Engineer
National Institute of Standards and Technology
eugenia.romantseva@nist.gov

Eugenia (Jane) Romantseva is a staff engineer supporting the Cellular Engineering Group at the National Institute of Standards and Technology (NIST). Jane leads NIST's efforts to develop measurement assurance and tools towards improving reproducibility in cell-free expression. Jane is also part of the collaborative effort to leverage automation, specifically the NIST Living Measurement Systems Foundry, for high throughput measurements to advance genetic sensor engineering and address challenges in scalability for engineering with living measurement systems. Jane received a B.S. in Mechanical Engineering from Boston University and a M.S. in Material Science and Engineering from Johns Hopkins University.

Jonathan Seppala, Ph.D.
Chemical Engineer
National Institute of Standards and Technology
jonathan.seppala@nist.gov

Dr. Jonathan Seppala leads the Polymer Additive Manufacturing and Rheology Project, developing multi-modal and in situ measurements that enable control over the complex non-equilibrium material dynamics that characterize soft matter processing. His current research uses infrared thermography, rheology, polarized light, fracture mechanics, and neutron and x-ray reflectivity and scattering to study the polymer physics of thermoplastic additive manufacturing processes. Jonathan earned a B.S. in Chemical Engineering from Michigan Technological University and a Ph.D. in Chemical Engineering from Michigan State University studying the rheology and thermodynamics of polymer nanocomposites. Following his Ph.D., Jonathan worked as a Postdoctoral Researcher studying thin film self-assembly of block copolymers and equilibrium dynamics of amphiphilic micelles at the University of Delaware. Before joining the Additive Manufacturing and Rheology Project, Jonathan studied ballistic witness materials and shear thickening fluids as part of NIST's Personal Body Armor Project.

Rohan Shirwaiker, Ph.D.
Professor
North Carolina State University
rashirwa@ncsu.edu

Dr. Shirwaiker is a Professor and Pleasant Faculty Scholar of Industrial & Systems Engineering and Associate Director of the Comparative Medicine Institute at NC State University. His research program focuses on the development and optimization of manufacturing processes and quality monitoring techniques to create and assess engineered tissues for a variety of biomedical and cellular agriculture applications. His research has been supported by the NSF, NIH, DOD, and the industry. Shirwaiker is a recipient of the NSF CAREER Award, SME Outstanding Young Manufacturing Engineer Award, and IISE Manufacturing & Design Outstanding Young Investigator Award. He currently serves on different boards and committees of IISE, SME, ASME, and ASTM.

Dielectric spectroscopy for in line monitoring of engineered tissue constructs

Abstract: This talk will highlight the application of dielectric spectroscopy, which leverages the responses of living cells to alternating electric fields, for the monitoring of engineered tissue constructs. Examples of mapping of dielectric parameters to critical quality attributes of constructs under scenarios ranging from static culture of cell-seeded scaffolds to perfusion bioreactor-based maturation of bioprinted constructs will be presented. Application of machine learning for more effective dielectric data analysis and decision-making will also be discussed.

Carl G. Simon, Jr., Ph.D.
Biologist
National Institute of Standards and Technology
carl.simon@nist.gov

Dr. Simon is a biologist in the Biomaterials Group at the National Institute of Standards & Technology. He leads projects on characterization of tissue engineered medical products, cell-material interactions and tissue engineering scaffolds. Dr. Simon is active in the Society for Biomaterials and is Chair of ASTM Committee F04.43 "Cells and Tissue-Engineered Constructs" where documentary standards are being advanced to support the development of medical products.

Optical Coherence Tomography Imaging for Label-Free Measurement of Cell Viability in Scaffolds

Abstract: In the field of tissue engineering, 3D scaffolds and cells are often combined to yield constructs that are used as therapeutics to restore tissue function in patients. Viable cells may be required to achieve the intended mechanism of action for the therapy, where the live cells may build new tissue or may release factors that induce tissue regeneration. Thus, there is a need to reliably measure cell viability in 3D scaffolds as a quality attribute of a tissue-engineered medical product. Here, we developed a label-free, 3D optical coherence tomography (OCT) method to image large sample volumes (1 mm^3) to quantitatively assess cell viability and distribution within scaffolds. OCT imaging was used to assess a model scaffold-cell system consisting of a polysaccharide-based hydrogel seeded with human Jurkat cells. Four test systems were used: i) hydrogel seeded with live cells, ii-iii)hydrogel seeded with heat-shocked or fixed dead cells and iv) hydrogel without any cells. OCT images revealed time-dependent changes in the refractive index (RI) within live cells that were due to intracellular movement of organelles (referred to as speckling patterns). The time-dependent changes in RI (speckle patterns) were not observed for hydrogels without cells or with hydrogels loaded with dead cells. The changes in speckle patterns were used to generate live-cell contrast by image subtraction where objects with large changes in RI were binned as live cells. When using 3D OCT imaging to count live cells within a gel volume, the results were within 13% of the expected value derived from the number of live cells that were seeded into the gels. Additionally, the 3D distribution of live cells was mapped within a hydrogel scaffold to assess the uniformity of their distribution across the volume. These results demonstrate a label-free method to assess the spatial distribution of live cells within a 3D scaffold that may be useful for assessing tissue-engineered medical products.

Kaiming Ye, Ph.D.
Distinguished Professor, Chair of the BME Department
Director, Center of Biomanufacturing for Regenerative Medicine, Binghamton University
kye@binghamton.edu

Kaiming Ye is a SUNY Distinguished Professor, Chair of the BME Department, and Director, Center of Biomanufacturing for Regenerative Medicine at Binghamton University, SUNY. He is Fellow of BMES and AIMBE. He is past Chair of the Council of Chairs of Biomedical Engineering and a funding Chair of BMES Advanced Biomanufacturing SIG (ABioM-SIG). He has contributed significantly to national policy-making in science and engineering. During his tenure at NSF, he directed a biomedical engineering program. He was a member of the Interagency Workgroup for Neuroscience, Interagency Modeling and Analysis Workgroup, and Multiagency Tissue Engineering and Regenerative Medicine Workgroup. He served on NIH/NIDDK Rebuilding Kidney Consortium's Advisory Committee. Ye pioneered human islet organoid development from pluripotent stem cells (PSCs). His group is the first one demonstrated the feasibility of generating the whole human islets from PSCs. His work in 3D tissue bioprinting was featured in Prism in 2015. He is one of the pioneers in developing fluorescence resonance energy transfer nanosensors for continuous glucose monitoring. As a researcher, he has secured more than $43 million grants as PI or co-PI. He has published more than 100 research articles, book chapters, and reports. He has chaired and co-chaired more than 18 international and national conferences and delivered more than 150 keynote/plenary/invited speeches in international and national conferences and graduate seminars in more than 100 universities.

A.3. Workshop Chairs and Organizing Committee

Workshop Co-Chairs

- Dr. Greta Babakhanova, Physicist, Material Measurement Laboratory, National Institute of Standards and Technology (NIST) | USA
- Dr. Carl G. Simon, Jr. Biologist, Material Measurement Laboratory, National Institute of Standards and Technology (NIST) | USA

Workshop Organizing Committee

- Dr. Guillermo Ameer, Director, Center for Advanced Regenerative Engineering, Northwestern University | USA
- Dr. Leanne Friedrich, Material Scientist, Material Measurement Laboratory, NIST | USA
- Dr. Dawn Henke, Senior Scientific Program Manager, Standards Coordinating Body | USA
- Dr. Jay Hoying, Chief Scientist, Advanced Solutions | USA
- Dr. John Huang, Founder & CEO, TheWell Bioscience, Inc. | USA
- Dr. Taneka Jones, Medical Science Liaison, Vericel | USA
- Dr. Mary Clare McCorry, Director, Technology and Process Development, ARMI BioFabUSA, | USA
- Marian Piekarczyk, Sr. Technical Specialist-Regenerative Medicine and Cell Biology, Thermo Fisher Scientific | USA
- Ms. Laura Pierce, Biomedical Engineer, Material Measurement Laboratory, NIST | USA
- Dr. Kimberlee Potter, Scientific Portfolio Manager for Restorative Medicine, U.S. Department of Veterans Affairs | USA
- Dr. Itedale Namro Redwan, Scientific Officer, CELLINK | Sweden
- Ms. Eugenia Romantseva, Engineer, Material Measurement Laboratory, NIST | USA
- Dr. Jonathan Seppala, Chemical Engineer, Material Measurement Laboratory, NIST | USA
- Dr. Rohan Shirwaiker, Professor, North Carolina State University | USA

A.4. Workshop Recordings and Presentations

Workshop recordings and presentations are available at the following links:

- Welcome Address (Recording)

  Sheng Lin Gibson, *NIST*

- Plenary: Manufacturing complex biologics for regenerative medicine (Slides | Recording)
  Jennifer H. Elisseeff, *Johns Hopkins University*

- Non-invasive non-contact real-time monitoring of cells within bio-reactors by direct imaging with optical coherence tomography (Slides | Recording)
  Naresh Menon, *ChromoLogic*

- Nondestructive Cell viability assessment using oxygen imaging (Recording)
  Mrignayani Kotecha, *O2M Technologies*

- Optical coherence tomography imaging for label-free measurement of cell viability in scaffolds (Recording)
  Carl Simon, *NIST*

- Cell Viability Panel Discussion (Recording)

- Advancing Drug Discovery with Biofabricated 3D Tissue Models (Recording)
  Marc Ferrer, *NCATS*

- Protein Sensing (Slides | Recording)
  Marcie Black, *Advanced Silicon Group*

- Non-invasive quantitative live cell imaging (Recording)
  Kersti Alm, *Phase Holographic Imaging*

- Cell Phenotype Panel Discussion (Recording)

- Dielectric spectroscopy for in line monitoring of engineered tissue constructs (Recording)
  Rohan Shirwaiker, North Carolina State University

- In situ volumetric imaging and analysis of 3D bioprinted constructs using optical coherence tomography (Recording)
  Adam Feinberg, *FluidForm*

- Characterization of tissue engineered medical products (Slides | Recording)
  Bao-Ngoc Nguyen, *FDA*

- Tissue Characterization Panel Discussion (Recording)

- Pre-workshop Survey Review (Recording)
  Carl Simon, *NIST*