

# **Sensor-Enabled Accelerated Engineering of Soft Materials**

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## **Abstract**

Many grand societal challenges are rooted in the need for new materials, such as those related to energy, health, and the environment. However, the traditional way of discovering new materials is basically trial and error. This time-consuming and expensive method can't meet the quickly growing requirements for material discovery. To meet this challenge, the government of the United States started the Materials Genome Initiative (MGI) in 2011. MGI aims at accelerating the pace and reducing the cost of discovering new materials. The success of MGI needs materials innovation infrastructure including data tools, computation tools, and experiment tools. The last decade has witnessed significant progress for MGI, especially with respect to hard materials. However, relatively less attention has been paid to soft materials. One important reason is the lack of experimental tools, especially characterization tools for high-throughput experimentation. This dissertation aims to enrich the toolbox by trying new sensor tools for high-throughput characterization of hydrogels. Piezoelectric-excited millimeter-sized cantilever (PEMC) sensors were used in this dissertation to characterize hydrogels. Their capability to investigate hydrogels was first demonstrated by monitoring the synthesis and stimuli-response of composite hydrogels. The PEMC sensors enabled in-situ study of how the manufacturing process, i.e. bulk vs. layer-by-layer, affects the structure and properties of hydrogels. Afterwards, the PEMC sensors were integrated with robots to develop a method of high-throughput experimentation. Various hydrogels were formulated in a well-plate format and characterized by the sensor tools in an automated manner. High-throughput characterization, especially multi-property characterization enabled optimizing the formulation to achieve tradeoff between different properties. Finally, the sensor-

based high-throughput experimentation was combined with active learning for accelerated material discovery. A collaborative learning was used to guide the high-throughput formulation and characterization of hydrogels, which demonstrated rapid discovery of mechanically optimized composite glyogels. Through this dissertation, we hope to provide a new tool for high-throughput characterization of soft materials to accelerate the discovery and optimization of materials.

# **Sensor-Enabled Accelerated Engineering of Soft Materials**

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## **General Audience Abstract**

Many grand societal challenges, including those associated with energy and healthcare, are driven by the need for new materials. However, the traditional way of discovering new materials is based on trial and error using low throughput computational and experimental methods. For example, it often takes several years, even decades, to discover and commercialize new materials. The lithium-ion battery is a good example. Traditional time-consuming and expensive methods cannot meet the fast-growing requirements of modern material discovery. With the development of computer science and automation, the idea of using data, artificial intelligence, and robots for accelerated materials discovery has attracted more and more attention. Significant progress has been made in metals and inorganic non-metal materials (e.g., semiconductors) in the past decade under the guidance of machine learning and the assistance of automated robots. However, relatively less progress has been made in materials having complex structures and dynamic properties, such as hydrogels. Hydrogels have wide applications in our daily lives, such as drugs and biomedical devices. One significant barrier to accelerated discovery and engineering of hydrogels is the lack of tools that can rapidly characterize the material's properties. In this dissertation, a sensor-based approach was created to characterize the mechanical properties and stimuli-response of soft materials using low sample volumes. The sensor was integrated with a robot to test materials in high-throughput formats in a rapid and automated measurement format. In combination with machine learning, the high-throughput characterization method was demonstrated to accelerate the engineering and optimization of several hydrogels. Through this dissertation, we hope to provide new tools and methods for rapid engineering of soft materials.

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# 1 Introduction

## 1.1 Background and Motivation

The traditional method to discover and optimize materials is trial and error which is a time-consuming and expensive process. It usually takes years even decades to discover and commercialize new materials, Teflon and lithium-ion batteries are good examples.<sup>1</sup> To meet new global challenges, like energy crisis and environmental protection, it's necessary to improve the way of material discovery. In 2011, the government of the United States started the Materials Genome Initiative (MGI) which aims at accelerating the pace and reducing the cost of discovering, manufacturing, and deploying advanced materials. The success of MGI needs materials innovation infrastructures, including data tools, computational tools, and experimental tools.<sup>2</sup> More than a decade has passed since the initiation of MGI, and a lot of progress has been made. Significant progress has been made in hard materials by far, including metal alloys and inorganic non-metal materials. These hard materials have wide applications in photovoltaics, batteries, catalysts, microelectronics, and so on. On the other hand, relatively less attention has been paid to soft materials.<sup>3</sup> Partly because of the complexity of soft materials arise from the hierarchy of structures and dynamic properties. Partly due to the lack of experimental tools for fast synthesis and characterization. In other words, the scarcity of tools for high-throughput experimentation limited the advance of MGI in soft materials. Hydrogels are important soft materials, which have important applications ranging from pharmaceuticals, tissue engineering, to soft robotics. There are several methods that can be used to prepare hydrogels in a high-throughput manner, such as microfluidics, microarray, and gradient.<sup>4</sup> However, few tools are available for high-throughput characterization of hydrogels. The motivation of this research is to try new techniques for high-

throughput characterization of soft materials (e.g., hydrogels). We hope to enrich the toolbox of high-throughput experimentation for soft materials to accelerate the discovery of new materials.

## **1.2 Research Objectives**

The overall objective of this dissertation is to expand the toolbox of high-throughput characterization of soft materials, thus, to accelerate materials discovery. While soft materials are a broad concept, we focus on hydrogels in this dissertation because of their availability and wide applications. The overall objective is achieved by dividing it into three small tasks.

Task one is to demonstrate the tool has the capability to characterize hydrogels. In this task, we try to apply piezoelectric-excited millimeter-sized cantilever (PEMC) sensors for in-situ and real-time monitoring of the hydrogelation and stimuli response of composite hydrogels. In addition, we expect the sensor to investigate how the manufacturing methods (bulk vs. layer-by-layer) affect the structure and properties of hydrogels.

Task two is to apply the PEMC sensors for high-throughput characterization of hydrogels. We expect the small size of PEMC sensors to enable them to be integrated with robots for automated measurement. Together with automated dispensing and mixing, we plan to develop a well plate-based format for high-throughput synthesis and characterization of hydrogels.

Task three is to combine sensor-based high-throughput experimentation with active learning to accelerate material discovery. In this task, we aim at fulfilling a closed-loop of accelerated materials discovery by introducing a collaborative learning to guide the high-throughput experimentation. We hope to give an example of how the sensor-based experimental tools and computational tools together to achieve the goal of MGI in soft materials.

### **1.3 Organization of the dissertation**

The remainder of the dissertation is organized as follows. The introduction of MGI and the progress in high-throughput experimentation are reviewed in Chapter 2. The first task of applying PEMC sensors to investigate the hydrogelation of thermal-responsive hydrogels is discussed in Chapter 3. The second task of developing sensor-based high-throughput characterization is demonstrated in Chapter 4. And the last task of combining the sensor-based high-throughput experimentation with active learning to accelerate material discovery is discussed in Chapter 5. Finally, the contribution of this dissertation and some future research directions is discussed in Chapter 6.

## 2 Literature Review

### 2.1 Materials Genome Initiative

Materials are the cornerstone of the world. From the prehistoric discovery of bronze and steel to the twentieth century invention of semiconductor materials and synthetic polymers, new materials always play important roles for vast transformations in human civilization. Today, materials innovations are still the key to overcoming global challenges such as climate change, energy crisis, human health, and poverty. However, it usually takes 10 – 20 years to incorporate a new material from initial research to first use, the development of lithium-ion battery is a recent example.<sup>1</sup> One reason it takes such a long time from material discovery to market is the continued reliance of materials research and development on scientific intuition and trial and error experimentation. Another reason is that current material deployment needs a series of continuous processes, including discovery, development, property optimization, systems design and integration, certification, manufacturing, and deployment. It comprises seven discrete states but lacks feedback between different phases. A new paradigm of material research and development is needed to meet fast-growing requirements for new materials.

Inspired by the human genome, the idea of high-throughput, data-driven approach to materials discovery was developed in the early 2000s.<sup>5</sup> After many efforts of some talented scientists the U.S. government noticed this idea and started the Materials Genome Initiative (MGI) in 2011. The MGI is a national effort to discover, manufacture, and deploy advanced materials twice as fast and at a fraction of the cost compared to traditional methods. It requires collaborations between material scientists and computation scientists to contribute to materials innovation infrastructures including computational tools, digital data, and experimental tools.<sup>2, 3</sup>

## 2.2 High Throughput Experimentation for Material Discovery

Computational simulations and models have demonstrated can improve the efficiency of material research and a lot of progress has already been made in this aspect. Experiments are still needed to validate these models because materials development is a complex, multi-dimensional problem. Therefore, High-throughput experimentation (HTE) becomes an essential part of MGI. In this section, we will have a brief introduction about the progress of HTE for materials discovery.

The idea of accelerating materials development with rapid experiments can be traced back to more than half a century ago.<sup>6</sup> In 1970, Hanak introduced an integrated materials-development workflow which was widely regarded as the earliest representative article for high-throughput materials research.<sup>7</sup> After decades of effort, HTE has attracted more and more attention. HTE, sometimes is also called “combinatorial materials screening” or “combinatorial and high-throughput (CHT) experimentation”,<sup>8</sup> is a process of parallelly synthesize a lot of samples (“library”) that contains variations of the materials parameter of interest (e.g., composition), and rapidly characterize the structural, physical, chemical, and any other property of interest of the material library.<sup>3, 9</sup> HTE has several advantages over traditional approaches to material development. First, HTE is generally faster and less expensive. Traditional trial and error methods can only study one parameter at one experiment while HTE can continuously change one or maybe more variables in one batch of experiments, which drastically reduces the number of experiments and saves a lot of time. HTE also reduces costs by using smaller samples for measurements and by reducing unnecessary waste (e.g., leftovers) in the format of parallel experiments. Furthermore, HTE carries out all experiments on the same library sample with the same manufacturing/characterization tools within a short time so that most systematic errors could be eliminated, thus the generated data would be more reliable and comparable. In addition, HTE

generates a lot of data which is a huge resource for computer scientists and data scientists to build models, predict trends, and screen the “sweet spot”. With the rapid development of artificial intelligence/machine learning, this advantage of HTE over traditional material development approaches has attracted more and more attention.<sup>10-15</sup> Thanks to these advantages, HTE has been used for materials substitution and experimental verification of materials properties predicted by modeling and simulation, in addition to materials discovery, screening, and optimization. The success of HTE in pharmaceutical industry is a good example.<sup>16, 17</sup> Now HTE has spread to many other fields, such as sensor materials and organic coating.<sup>18-20</sup>

The key of HTE is developing tools and methods for high-throughput material fabrication and characterization. Although there are a lot of traditional tools available, they are usually not applicable for HTE. Many efforts are still needed to invent new tools or to modify traditional ones more suitable for high-throughput applications, either the format, speed, and accuracy for materials fabrications/measurements or the cost of the equipment. Despite these challenges, a lot of progress has been made in the past two decades, especially hard materials which have wide applications for electronic, magnetic, optical, and energy-related fields.<sup>9</sup> Based on the category of materials, various high-throughput sample preparation methods have been reported.<sup>3, 8, 9, 21</sup> For metal alloy samples, they are mostly prepared in the format of gradient thin films, and thin-film vapor deposition (TFVD) which is based on physic vapor deposition (PVD) is the most popular method to fabricate composition gradient libraries.<sup>21</sup> One of the most common ways is co-sputtering. In this method, different resources are used at the same time to deposit a film on a substrate, and the composition gradient is controlled by adjusting the location and angle without shutter.<sup>22-24</sup> With the assistance of a shutter, a composition gradient library can also be produced by using different single deposition sources with the shutter moving over time.<sup>25, 26</sup> Another derived sputtering

method can even prepare discrete thin film library by using masks.<sup>27, 28</sup> Some non-sputtering methods also have been proposed, such as additive manufacturing, diffusion multiples, etc.<sup>29-32</sup> In addition, some multimetallic nanoparticles were also prepared in high-throughput manners although they are less common than thin films.<sup>33, 34</sup> For inorganic non-metallic materials, there are many high-throughput preparation methods reported too. TFVD is also the most popular method of high-throughput sample preparation. Chemical vapor deposition (CVD) as well as PVD are both used for high-throughput preparation of inorganic non-metallic materials. Compared with metal alloys, there are more reports of high-throughput preparation of discrete samples. Inkjet printing is another commonly used method for high-throughput preparation of inorganic non-metallic samples, such as fluorescent materials and catalysts.<sup>35-37</sup> In addition to these general methods, some specific methods also have been developed for specific applications. For instance, microfluidic technologies/flow chemistry/combinatorial electrochemistry platforms have been applied to develop catalysts and electrode materials.<sup>38-40</sup>

Another important part of HTE is high-throughput characterization. For hard materials, many tools have been used to characterize different properties in a high-throughput manner. For composition, many methods have been reported including Infrared spectroscopy (IR), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), Energy Dispersive X-Ray Analysis (EDX), X-ray fluorescence (XRF), and X-ray diffraction (XRD). Specially, XRD can characterize crystal structure as well as composition.<sup>41-45</sup> For conductivity and thermoelectricity, the most widely used method is the four-point probe.<sup>46-50</sup> For mechanical properties, nanoindentation is currently the representative method which can measure modulus and hardness.<sup>51-53</sup> For electrocatalytic and photoelectrochemical properties, scanning electrochemical microscopy (SECM) or scanning photoelectrochemical microscopy (SPECM) were reported.<sup>54, 55</sup> Although these techniques have

been used for conventional characterization, a lot of efforts are behind the modification to make them meet the needs of HTE. For example, Traditional XRD can't be used for high-throughput characterization until its resolution, brightness, and rate of data collection are improved.

## **2.3 High-throughput Experimentation for Hydrogels**

In the previous part we introduced the history of HTE and briefed the progress of HTE for hard materials. However, less attention has been given to soft materials than hard materials due to their complex hierarchy structures and dynamic behaviors. In this section we will introduce the current progress of HTE in polymers. Polymer materials have wide applications, and HTE has been applied for polymer synthesis (polymer solution), polymer hydrogels, polymer composites, and polymer films.<sup>56-59</sup> Here we only focus on polymer hydrogels, more information may refer to other good reviews.<sup>8, 60</sup>

### **2.3.1 High-throughput Synthesis of Hydrogels**

There are several methods that have been reported for high-throughput synthesis of hydrogels, and some representative publications are summarized in Table 2-1. Basically, there are three main methods for high-throughput preparation of hydrogel samples. The first common method is (micro)array. In this method, the monomer/precursor or polymer solutions are usually transferred to the substrate by non-contact printing (e.g., inkjet printing) or contact printing. For multi-component hydrogels, one component is first printed followed by overprinting of another component. Thus, the composition of the hydrogel can be controlled by adjusting the number of droplets of each component. For example, the initiator, monomer one, monomer two, and the activator could be printed orderly.<sup>61</sup> Sometimes, the substrates can be pre-treated to improve production of the array. For instance, Rosenfeld et al. developed a high-throughput array platform by patterning glass slides with hydrophilic regions separated by superhydrophobic liquid-

impermeable barriers to inhibit cross-contamination.<sup>62</sup> With the development of printers, the droplet can be as small as picoliter which gives high precise control of the volume and composition.<sup>63</sup> However, polymer solutions in (micro)array are usually mixed only by diffusion due to its small volume, which limited the application of viscous polymers. Well Plate is another spatial resolution method which is sometimes treated as a variant of (micro)array. Like (micro)array, printing technologies are also the main method to formulate hydrogel samples in well plate although manually pipetting is also acceptable. Compared to (micro)array, well plate has a larger volume that robot-assisted mixing and high-throughput characterization become possible.<sup>64, 65</sup>

Another high-throughput method to generate hydrogel samples is gradient. Different from spatial methods, gradient methods give continuous bulk hydrogel samples. There are two basic techniques to get gradient samples. The first one is (Micro-)fluidic gradient generation. In this technique, the gradient is usually generated by controlling the flow of each composition with pumps. Smart design of microfluidic device can also get spatial gradients by stepwise separation and remixing of precursor solutions in a progressive manner. Other methods to generate gradient include thermal cycles and light exposure.<sup>66, 67</sup> Another technique to generate gradient samples is convection-driven microfluidic mixing. This technique is based upon the dispersion effect, which can be achieved by back and forward pumping of two fluid plugs within a microfluidic channel.<sup>68</sup> Hydrogel samples obtained by gradient technique consist of a gradual compositional change between two or more parameters. However, this compositional change does not have to be linear as exponential, sigmoidal and radial gradients have been fabricated.<sup>4</sup> Due to the continuously changing nature of the gradient samples, it's difficult to isolate the optimal composition.

A third method for high-throughput experimentation of hydrogels is microfluidics. Microfluidics has long been used for high-throughput synthesis of hydrogels which can be summarized in many good reviews.<sup>69, 70</sup> While many reported applications only use microfluidics to generate uniform hydrogel microparticles, this method enables adjusting the properties of hydrogels continuously. For example, Kumachev et al. obtained a series of microbeads with different elasticity by controlling the flow rate of agarose having different concentrations.<sup>71</sup> Hydrogels fabricated by microfluidics have been widely used for bioengineering, such as cell encapsulation and tissue engineering.

In addition to the three methods mentioned above, some new methods emerged recently. One is using automated robots for high-throughput formulation of hydrogels. For example, Xu et al used a commercial high-throughput robotics system Tecan Evo 200 to formulate injectable hydrogels in 96-well plates.<sup>65</sup>

**Table 2-1** Representative Publications of High-throughput Experimentation for Hydrogels

| Material                                                                                          | Format     | HT Sampling         | Properties Characterized | HT Characterization | Applications | Ref. |
|---------------------------------------------------------------------------------------------------|------------|---------------------|--------------------------|---------------------|--------------|------|
| Acrylamide, Dimethylacrylamide, etc.                                                              | Microarray | Inkjet Printing     | Appearance               | Microscopy          | N/A          | 72   |
| N-isopropylacrylamide etc.                                                                        | Microarray | Inkjet Printing     | Cell growth              | Microscopy          | Cell culture | 61   |
| poly(ethylene glycol) dimethacrylate, 2-(N-3-sulfopropyl-N,N-dimethyl ammonium)ethyl methacrylate | Microarray | Inkjet Printing     | Degradability            | Microscopy          | N/A          | 62   |
| Poly(ethylene glycol)                                                                             | Microarray | Automated Pipetting | Stiffness Degradability  | N/A                 | Cell culture | 64   |
| PEGDMA, PHEMA, Poly(sulfobetaine methacrylate)                                                    | Microarray | Inkjet Printing     | Swell, Drug loading      | Imaging             | Drug Loading | 63   |

|                                                                         |                |                             |                                          |                          |                      |    |
|-------------------------------------------------------------------------|----------------|-----------------------------|------------------------------------------|--------------------------|----------------------|----|
| PEG (polyethylene glycol), RGD (arginine-glycine-aspartic acid) peptide | Microarray     | Photomask                   | RGD density                              | Microscopy               | Cell culture         | 73 |
| Gellan Gum, Gelatin Methacryloyl                                        | Gradient       | Fluidic gradient generation | Cell differentiation                     | Microscopy               | Cell culture         | 74 |
| Poly(propylene), poly(styrene)                                          | Gradient       | Fluidic gradient generation | Composition, Residence time distribution | Pulse tracer experiments | Film                 | 75 |
| Agarose                                                                 | Microparticle  | Microfluidics               | Elasticity                               | N/A                      | Cell encapsulation   | 71 |
| Poly(ethylene glycol)                                                   | Microparticle  | Microfluidics               | Cross-linking density                    | Microscopy               | Cell culture         | 76 |
| POEGMA etc.                                                             | 96 well-plate  | Automated Robot             | Swelling, Mechanics, Degradation         | Indentation              | Drug Release         | 65 |
| Polysulfobetaine                                                        | Home-made mold | Microfluidics               | Self-healing                             | N/A                      | Flexible Electronics | 77 |

### 2.3.2 High-throughput Characterization of Hydrogels

Although there are several high-throughput methods available for hydrogel synthesis, the bottleneck is high-throughput characterization. One high-throughput characterization method for hydrogels is microscopy. It can be used to characterize the distribution of each component in the formulated hydrogels by introducing dyes.<sup>62, 74</sup> Microscopy is also used to indirectly characterize the property of hydrogels through studying their interactions with cells after staining.<sup>73</sup> For other properties of hydrogels (e.g., composition, morphology), only some representative samples of the synthesized hydrogels are usually selected for characterization by traditional methods due to heavy workload.<sup>63, 73, 75</sup> Recently, Xu et al reported using a commercialized indentation machine to high throughput characterize the mechanical property of hydrogels.<sup>65</sup> Overall, the degree of automation is still low for high-throughput characterization of hydrogels and more characterization tools are needed to speed up the research in this field.

## 2.4 Artificial Intelligence and HTE

The success of MGI needs data tools, computational tools, and experimentation tools. While this dissertation mainly focusses on experimental tools, it also deserves to mention the other two aspects because these tools don't work independently. The emerging self-driving laboratory is an example of how the combination of artificial intelligence and automated hardware accelerates materials discovery. Many excellent reviews have given good summaries and perspectives about the role of data and machine learning in MGI.<sup>12, 13, 15, 78</sup> Here, I only give one example of combining machine learning and automated high-throughput experimentation to accelerate the discovery of soft materials. Rooney et al. reported using a self-driving laboratory to accelerate the optimization of adhesive materials.<sup>79</sup> They designed a modular robotic platform that can perform a complex sequence of eight tasks including surface preparation, test specimen assembly, and bond strength evaluation. The obtained data from previous experiments can be put into a Bayesian optimizer to choose which formulations to test next, thus forming a closed loop. This self-driving laboratory can run without human supervision and test up to 50 independent samples before restocking.

### **3 Comparison of Bulk- vs. Layer-by-layer-cured Stimuli-responsive PNIPAM-Alginate Hydrogel Dynamic Viscoelastic Property Response via Embedded Sensors**

While stimuli-responsive hydrogels are now being widely investigated, such for additive manufacturing applications, it remains a challenge to continuously monitor the dynamic response of their material properties to stimuli using traditional characterization methods. Here, we report that dynamic-mode piezoelectric milli-cantilever sensors enable real-time monitoring of the viscoelastic response of bulk- and layer-by-layer (LBL)-cured composite poly(N-isopropylacrylamide) (PNIPAM)-alginate hydrogel constructs to thermal changes across the 25 – 37 °C temperature range. Scanning electron microscopy and sensing studies revealed that the network structure and viscoelastic response of ionic-covalent entanglement composite PNIPAM-alginate hydrogel constructs are dependent on the hydrogel processing method. Composite PNIPAM-alginate constructs fabricated using LBL curing exhibited relatively increased responsiveness compared to bulk-cured constructs in terms of the magnitude of thermal stimulus-driven shear storage modulus change, suggesting opportunities for additive manufacturing applications. In summary, we show that sensors in combination with traditional characterization methods enable the study of dynamic process-structure-rheological property relations of stimuli-responsive soft materials and real-time monitoring of material rheological properties using a low-sample volume measurement format.

#### **3.1 Introduction**

Additive manufacturing using stimuli-responsive hydrogels has enabled emerging applications that require dynamic property or shape change, including soft robotics and shape memory actuators.<sup>80-82</sup> Hydrogels are viscoelastic materials composed of a three-dimensional (3D) polymer

network that contain high amounts of water.<sup>83</sup> Stimuli-responsive hydrogels exhibit temporal changes in structure, properties, and shape in response to environmental stimuli.<sup>84-87</sup> Various stimuli have been examined for actuation of hydrogel response, including physical (e.g., temperature, electric field, magnetic field, light), chemical (e.g., pH), and biological stimuli (e.g., affinity ligands, enzyme substrates). For example, poly(N-isopropylacrylamide) (PNIPAM)-water mixtures, which exhibit a lower critical solution temperature (LCST) of 32 °C, are widely investigated thermo-responsive hydrogels.<sup>88</sup> In addition to hydrogels composed of a single polymer component, composite hydrogels have also received considerable attention based on their unique properties that may arise from interactions among different polymer species. Understanding of process-structure-property relations of composite hydrogels remains an active area of research. Composite hydrogels have been already utilized in a range of smart applications. Bakarich *et al.* fabricated a smart valve of PNIPAM-alginate hydrogels using microextrusion printing to control water flow rate.<sup>89</sup> The valve closed and opened upon exposure to hot and cold water, respectively, based on the thermal expansion of the hydrogel over the 20 - 60 °C temperature range. To date, various additive manufacturing processes have utilized PNIPAM and composite PNIPAM hydrogels to fabricate layer-by-layer (LBL) constructs in various geometries, such as dumbbell-shaped structures, flower biomimetics, tubes, and so on.<sup>90-94</sup> However, there remains a need to investigate potential process-material interactions that may affect the quality of layered hydrogel constructs (i.e., fabricated by LBL curing based processing methods, such as additive manufacturing processes) in terms of resultant properties and stimuli response. In particular, new methods for real-time monitoring of material dynamic response to stimuli could yield new insights into the design of stimuli-responsive hydrogels.

While a variety of methods have been established to characterize the structure and properties of hydrogels, relatively few are able to assess the real-time response of stimuli-responsive hydrogels. For example, while compressive testing is commonly utilized for characterization of hydrogel modulus,<sup>95-97</sup> the method involves relatively long manual sample preparation and testing times. In addition to mechanical properties, the passive swelling response of hydrogels in water based on weight or volume change measurements provides a common method of characterizing hydrogel microstructure, including density of crosslinking, average molecular weight between crosslinks, and mesh size.<sup>98-100</sup> Scanning electron microscopy (SEM) can also provide insight into the microstructure and morphology of hydrogels based on image data.<sup>101-103</sup> Another method for characterizing hydrogel morphology is small-angle neutron scattering (SANS), which is useful for investigating the phase behavior, such as phase separation of PNIPAM.<sup>104-106</sup> More recently, confocal laser scanning microscopy (CLSM) has been applied to evaluate the structure of complex synthetic materials, including hydrogels.<sup>107</sup> Fluorescently stained hydrogels can be imaged without the need for sample freeze-drying enabling visualization of static and dynamic structure via time-lapse CLSM. For instance, Si et al. studied the effect of acrylic acid weight percentage on the pore size in poly(N-Isopropyl acrylamide-co-acrylic acid) microspheres, and Kubota *et al.* imaged the pH-responsive hydrogelation process of a peptide-grafted polymer.<sup>108, 109</sup> In addition to characterization of hydrogel mechanical properties based on uniaxial testing, characterization of hydrogel rheological properties is also commonly utilized in hydrogels materials research. For example, hydrogel rheological properties provide insight into gelation processes as well as hydrogel processability. Rheological properties can also provide insight to hydrogel microstructures.<sup>110-112</sup> For instance, Karvinen *et al.* quantified the average mesh size, crosslinking density, and average molecular weight of the polymer chain between neighboring crosslinks of

hydrazone crosslinked polysaccharide hydrogels by applying amplitude and frequency sweep measurements obtained by dynamic mechanical analysis (DMA).<sup>113</sup> Despite these advances, the ability to characterize the real-time response of hydrogel structure and properties in response to stimuli is still a bottleneck, especially using measurement formats that involve minimal sample preparation and small sample volumes.

Unlike the majority of traditional characterization methods, which are constrained by low throughput and time-to-results due to intensive manual sample preparation and lengthy characterization tests, sensor-based material characterization methods exhibit various advantages, including transducers that exhibit high sensitivity, continuous monitoring, reduction of required sample size and preparation time, and potentially improved reproducibility relative to methods that involve intensive manual sample preparation.<sup>114-116</sup> Quartz crystal microbalance with dissipation monitoring (QCM-D) technology, for example, has been effectively used to characterize the viscoelastic response and properties of PNIPAM hydrogel thin films. Changes in temperature-driven frequency and dissipation factor enabled monitoring of viscoelasticity and structure response of PNIPAM hydrogels during phase transition.<sup>117</sup> However, QCM-D is typically limited to characterization of thin-film structures. In addition, QCM sensors may exhibit form factors that are challenging to scale and integrate within practical material processes (e.g., additive manufacturing processes) for purposes of online process monitoring and control. High-speed atomic force microscopy made by Matsui *et al.* was reported to detect the size and morphology change of PNIPAM at the individual microgel level.<sup>118</sup> Haring et al showed that dynamic-mode piezoelectric milli-cantilever (PEMC) sensors enabled the characterization of gelatin and alginate hydrogel viscoelastic properties and continuous monitoring of sol-gel phase transitions.<sup>114</sup> Cesewski et al. further showed that high-order modes of PEMC sensors near 800 kHz enable

sensitive characterization of hydrogel viscoelastic properties.<sup>115</sup> Using cantilever sensors for real-time monitoring, Singh et al designed a closed-loop to control photopolymerization process for the fabrication of photocurable hydrogels with controlled storage moduli.<sup>116</sup> Thus, it is of interest to examine the potential for PEMC sensors to characterize the real-time stimuli-response of bulk-cured vs. layered PNIPAM-based hydrogels.

Here, we show that dynamic-mode PEMC sensors facilitate real-time monitoring of multi-step composite ionic-covalent entanglement (ICE) PNIPAM-alginate hydrogel gelation processes and composite hydrogel response to temperature change. We further compare the difference in the network structure and viscoelastic properties of composite hydrogels fabricated by bulk- vs. LBL-curing processes. Hydrogel viscoelastic properties obtained by sensor data and cantilever fluid-structure interaction were benchmarked against DMA.

## **3.2 Materials and Methods**

### **3.2.1 Materials**

Alginic acid sodium salt (AlgNa), 2,2-dimethoxy-2-phenylacetophenone (DMPA), calcium chloride ( $\text{CaCl}_2$ ), N-isopropylacrylamide (NIPAM), and N,N'-methylenebisacrylamide (MBAA) were purchased from Sigma-Aldrich. Lead zirconate titanate (PZT-5A,  $72.4 \times 72.4 \times 0.127 \text{ mm}^3$ ) with nickel (Ni) electrodes was from Piezosystems (Woburn, MA). Glass cylinders and ethanol (200 proof) were from Fisher Scientific. Polyurethane (Fast-Drying) and epoxy (EA 1C-LV) were from Minwax and Loctite, respectively.

### **3.2.2 Fabrication of Piezoelectric-excited Millimeter Cantilever Sensors.**

Piezoelectric milli-cantilever (PEMC) sensors ( $n = 5$ ) were fabricated from Ni-electroded PZT as described in our other studies.<sup>114, 115, 119</sup> Briefly, PZT sheets were diced into chips ( $5 \times 1 \times 0.127 \text{ mm}^3$ ; American Dicing, Liverpool, NY). Insulated wires (30-gauge; copper) were soldered to the

top and bottom faces of the Ni electrodes on the distal end of the PZT layer. The soldered distal end of the chip, including solder points, was then potted in a glass cylinder (6 mm diameter) with a non-conductive epoxy resulting in a cantilever geometry ( $3 \times 1 \times 0.127 \text{ mm}^3$ ). Additional epoxy was applied on one side of the PZT to create an asymmetric anchor.<sup>119</sup> The sensors were subsequently coated with polyurethane via brush coating and allowed to cure at room temperature. Following polyurethane coating, the sensors were then coated with Parylene C ( $\sim 10 \text{ }\mu\text{m}$  thick) following vendor protocols (PDS 2010 Labcoter® 2, Specialty Coating Systems; Indianapolis, IN). Following Parylene C coating, the sensors were annealed for 1 hr at  $75 \text{ }^\circ\text{C}$ .

### **3.2.3 Real-time Sensor-based Monitoring of Hydrogel Gelation and Thermal Expansion**

The precursor solution for the composite ICE PNIPAM-alginate hydrogel was prepared by vigorously mixing alginate stock solution (2 wt.% of AlgNa/water), NIPAM, MBAA, DMPA stock solution (1%wt of DMPA/ethanol), and deionized water. The concentration of alginate and NIPAM in the final solution were 1 and 8 wt.%, respectively. The concentration of DMPA and MBAA in the final solution were 0.15 and 0.5%, respectively, with respect to the NIPAM monomer concentration. The solution used for NIPAM hydrogels exhibited the same formulation but did not contain alginate.

Gelation studies were performed in a custom aluminum mold (length  $\times$  width  $\times$  height =  $39 \times 21 \times 13 \text{ mm}^3$ ; see Figure SA - 1). The mold was immersed in a water bath ( $20 \text{ }^\circ\text{C}$ ) for temperature control. Prior to all experiments, the sensor was positioned 0.2 mm from the bottom of the mold using a manual micro-manipulator. The sensor resonant frequency ( $f$ ) and phase angle at resonance ( $\phi$ ) were continuously monitored and acquired with a vector-network analyzer with the impedance option (E5061b-005; Keysight) and a custom MATLAB program. The phase angle of the PEMC sensor, which is defined as  $\phi = \arctan[\text{Im}(Z)/\text{Re}(Z)]$ , where  $Z$  is the electrical impedance of the

piezoelectric layer of the PEMC sensor, should not be confused with the phase lag associated with the viscoelastic response of the surrounding material. The phase angle of an RC circuit is between  $-90$  and  $0^\circ$ , since the reactive capacitance ( $X_C$ ) is often defined as a negative value by convention (i.e.,  $X_C = -1/(\omega C)$ , where  $\omega$  is the angular frequency and  $C$  is the capacitance). Thus, since the phase angle is negative, the current leads the applied voltage in an RC circuit. As discussed, the phase angle of the PEMC sensor is different than phase lag ( $\delta$ ) between applied stress and resultant strain in the surrounding viscoelastic material that results from the oscillating cantilever, which is defined as  $\delta = \arctan[\text{Im}(G^*)/\text{Re}(G^*)] = \arctan[G''/G']$ , where  $G^*$  is the complex shear modulus of the surrounding material, here, a polymer solution or hydrogel. While the phase angle of an RC circuit, such as a PEMC sensor, ranges from  $-90$  to  $0^\circ$ ,  $\delta$  ranges from  $0$  and  $90^\circ$ . While  $\phi$  and  $\delta$  are different physical quantities as discussed, the change in  $\phi$  is governed by the motional changes in the circuit resistance, capacitance, and inductance, which are dependent on the surrounding material's viscoelastic properties (and thus,  $\delta$ ). Thus, it is possible to characterize changes in the surrounding material's viscoelastic properties (a mechanical system), by measuring changes in the frequency response of the electrical circuit formed by the PEMC sensor (an electromechanical system) that is mechanically coupled to the sensor through fluid-structure interaction effects.<sup>120</sup> Electrical impedance analysis was performed using a stimulus amplitude of 100 mV AC and zero DC bias across a frequency range ( $f \pm 10$  kHz).

Following stabilization of the sensor signals, bulk-cured hydrogels were prepared by adding 1 mL of precursor solution to the mold. Bulk-cured composite PNIPAM-alginate hydrogels were prepared by first photocuring NIPAM for 30 minutes (365 nm; UVGL-58; UVP) to form the PNIPAM network. The alginate network and resultant ICE composite PNIPAM-alginate hydrogel was subsequently formed by ionic crosslinking by depositing a 100  $\mu$ L droplet of calcium chloride

(500 mM) on the surface of the material approximately 5 mm from the submerged sensor. PNIPAM and alginate hydrogels served as the negative controls and were synthesized by the same methods. Four-layer LBL-cured composite PNIPAM-alginate hydrogel constructs were prepared by layer-by-layer (LBL) photocuring (250  $\mu$ L per layer) over the course of 80 min. Following the photocuring of each layer, the alginate network was ionically crosslinked by depositing a 100  $\mu$ L droplet of calcium chloride (500 mM) on the surface of the multi-layer construct approximately 5 mm from the submerged sensor. For investigation of hydrogel thermal response, the hydrogels were cycled from 25 to 37 to 25  $^{\circ}$ C.

### 3.2.4 Data Analysis

The resonance amplitude,  $A$ , (i.e., peak height) was calculated as the difference between phase angle at resonance ( $\phi$ ) and baseline phase angle off resonance ( $\phi_0$ ). For example, the resonance amplitude of the cantilever sensor in air was given as  $A_{Air} = \phi_{Air} - \phi_0$ . Thus,  $A_{Air}$ ,  $A_{Solution}$ ,  $A_{UV}$ , and  $A_{CaCl2}$  represented the change in  $\phi$  relative to the baseline value in air associated with the precursor solution, photocured hydrogel, and chemically-crosslinked hydrogel, respectively. To describe the PNIPAM gelation process, the percent change of the resonance amplitude caused by photocuring was calculated by  $\Delta A_{UV} = (A_{UV} - A_{Solution}) / A_{Solution} * 100$ . Similarly, the percent change of resonance amplitude caused by calcium chloride crosslinking was  $\Delta A_{CaCl2} = (A_{CaCl2} - A_{Solution}) / A_{Solution} * 100$ . The composite PNIPAM-alginate hydrogels required two sequential reactions for preparation. For comparison of composite PNIPAM-alginate and alginate hydrogels, the percent change of the second step (CaCl<sub>2</sub> crosslinking) relative to the first step (UV curing) was calculated as  $\Delta A_{CaCl2} = (A_{CaCl2} - A_{UV}) / A_{UV} * 100\%$ . We also define  $\Delta A_{UV} = (A_{UV} - A_{Air}) / A_{Air} * 100\%$  when comparing the gelation of bulk- vs. LBL-photocured hydrogels given the absence of  $A_{Solution}$  for the reaction. Significance testing was performed using a Student's t-test (Microsoft Excel).

### **3.2.5 Characterization of Hydrogel Low-Frequency Viscoelastic Moduli via Dynamic Mechanical Analysis**

Characterization of hydrogel low-frequency compressive storage and loss moduli ( $E'$  and  $E''$ , respectively) was done using a dynamic mechanical analyzer (Q800; TA Instruments). Hydrogel specimens were prepared using a custom mold (diameter = 10 mm; height  $\sim$  5 mm). All measurements were acquired at 25 °C by application of a 15  $\mu$ m periodic displacement at a constant frequency (1 Hz) and 5 mN preload force in the compression mode. The compressive moduli were converted to shear moduli ( $G'$  and  $G''$  at 1 Hz) using the relation  $G = E/[2(1+\nu)]$ , where  $\nu$  is the Poisson ratio assuming  $\nu = 0.5$ , which is a reasonable assumption for hydrogels.<sup>121, 122</sup>

### **3.2.6 Characterization of Hydrogel High-Frequency Viscoelastic Moduli via Sensor Data and Fluid-Structure Interaction Models**

While low-frequency  $G'$  and  $G''$  (i.e.,  $G'$  and  $G''$  at 1 Hz) can be obtained from dynamic mechanical analysis,  $G'$  and  $G''$  at  $f$  can be obtained from cantilever fluid-structure interaction theory and experimentally measured  $f$  and quality factor ( $Q$ ) response. A detailed description of the fluid-structure interaction model and underlying assumptions for piezoelectric milli-cantilever sensors can be found in our previous work.<sup>114</sup>

### **3.2.7 Characterization of Hydrogel Network Structure via Scanning Electron Microscopy**

The network structures of the swollen hydrogels below and above the volume phase transition temperature (VPTT) of PNIPAM were characterized using variable-pressure scanning electron microscope systems (Hitachi SU-5000 and Hitachi SU-6600). Prior to mounting the membranes, each sample was first hydrated in deionized water for at least 24 hr. To image the network structure above the VPTT, the hydrogels were heated to 55 °C in a water bath for 4 hr before being flash frozen using liquid nitrogen; samples below the VPTT were not heated but were subjected to the same flash freezing procedure as those samples that were. All hydrogels (samples 5 mm  $\times$  15mm

× 20 mm) were then dried by lyophilization for 24 hr at -105 °C; (FreeZone; Labconco). Prior to inserting samples into the SEM, the samples were attached by conductive adhesive tape to an aluminum specimen holder and sputter-coated with a thin layer of platinum (~ 3 nm; Anatech LTD Hummer 6.2).

### **3.3 Results and Discussion**

#### **3.3.1 Real-time Sensor-based Monitoring of ICE Composite PNIPAM-Alginate Hydrogel Gelation**

Schematics associated with the PEMC sensor design, measurement format, crosslinking steps, and network structure of ionic-covalent entanglement (ICE) composite PNIPAM-alginate hydrogels are shown in Figure 3.1. As shown in Figures 3.1A and 3.1B, hydrogel viscoelastic property monitoring is performed by continuous monitoring of resonance in submerged piezoelectric millilever sensors. Sample (precursor solution) volumes of 250 – 1000  $\mu$ L were used in this study. PNIPAM monomer was first photocured to form a PNIPAM network. The alginate network was subsequently formed by chemical crosslinking with calcium chloride solution to create an ICE PNIPAM-alginate network (see Figure 3.1C). We note that other calcium-containing salts, which may exhibit different crosslinking rates and resultant hydrogel properties and characteristics (e.g., sample homogeneity), can also facilitate alginate crosslinking.<sup>123</sup> Here, we utilized calcium chloride based on its extensive use in additive manufacturing applications.<sup>124</sup> No phase separation was observed during reactions. The resultant composite PNIPAM-alginate hydrogels were transparent. We found that in the absence of temperature control (e.g., via water bath) that maintains the temperature below LCST, hydrogels appeared opaque due to the heat released during polymerization.

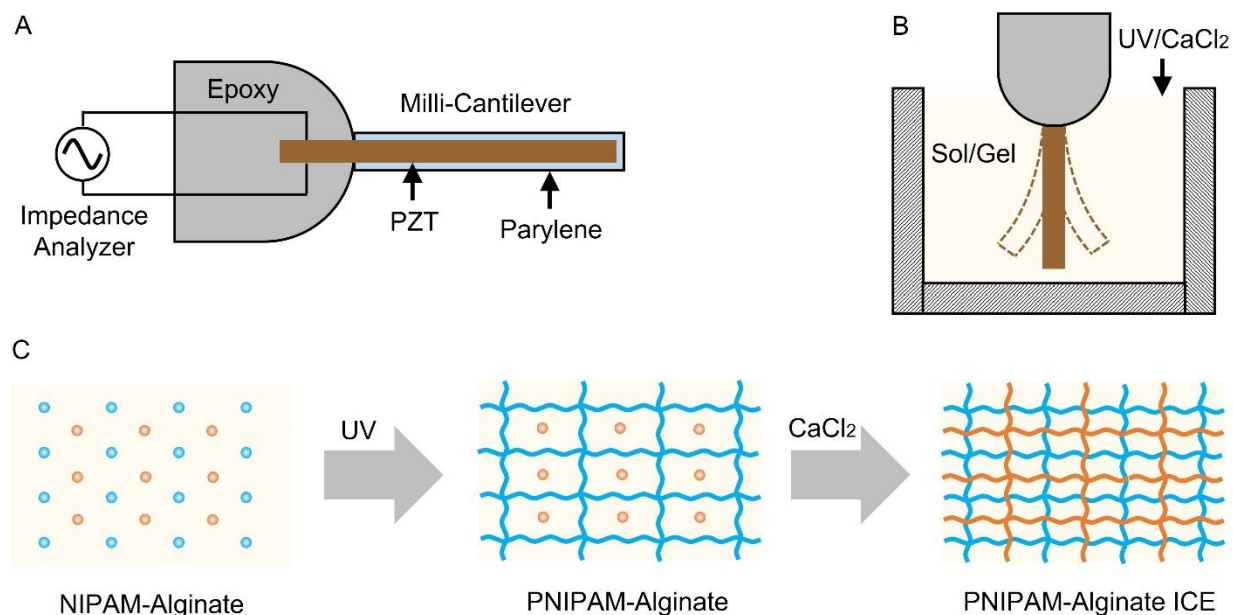


**Figure 3.1** Schematic of a PEMC sensor (A) and measurement format (B) for real-time monitoring ICE composite PNIPAM-alginate hydrogel assembly, gelation, and viscoelastic property response. C) Crosslinking process and network structure associated with ICE composite PNIPAM-alginate hydrogels.

Given composite PNIPAM-alginate hydrogels are formed by interpenetrating networks of PNIPAM and alginate, PNIPAM and alginate hydrogels served as controls for the study. As shown in Figures 3.2A-C, PEMC enabled characterization of NIPAM, alginate, and composite PNIPAM-alginate hydrogel gelation, respectively, via phase angle response over the 15 – 55 kHz frequency range ( $f_{air} = 40.75$  kHz). Submersion of the sensor in precursor solution caused a significant decrease of the  $\phi$ ,  $Q$ , and  $f$  based on a combination of fluid added mass and damping effects ( $f_{sol} = 31.7$  kHz). Gelation of the precursor solution by photocuring or chemical crosslinking resulted in a significant change in  $\phi$  (i.e., resonance amplitude), while only a minor shift in  $f$  was observed. This result is consistent with our previous study of sensor-based monitoring of alginate gelation.<sup>114</sup>

<sup>115</sup> Real-time monitoring of the composite PNIPAM-alginate hydrogel gelation process via

continuous tracking of  $\phi$  in comparison with control PNIPAM and alginate hydrogels is shown in Figures 3.2D-F. The  $f$  and  $Q$  responses are provided in Supporting Figure SA - 2. The formation of the PNIPAM network caused a relatively smaller  $\phi$  change than alginate network formation ( $19.2 \pm 1.3\%$  vs.  $52.7 \pm 3.5\%$ ;  $n = 5$ ;  $p = 0.05$ ). As shown in Figures 3.2G-I, the real-time sensor data ( $f$  and  $Q$ , which is correlated with  $\phi$ ) facilitates real-time monitoring of the hydrogel viscoelastic property changes during the two-step reaction based on a fluid-structure interaction model of Mather *et al.*:<sup>114-116, 120</sup>

$$g_1 = \frac{\pi b_2 G''}{2\omega} + \frac{\pi}{4\sqrt{2}} \sqrt{\rho} b \left[ (b_1 - a_2) \sqrt{\sqrt{G'^2 + G''^2} + G'} + (a_2 + b_1) \sqrt{\sqrt{G'^2 + G''^2} - G'} \right] \quad (1)$$

$$g_2 = \frac{\pi}{4} a_1 \rho b^2 + \frac{\pi}{2} b_2 \frac{G'}{\omega^2} + \frac{\pi}{4\sqrt{2}} \frac{\sqrt{\rho} b}{\omega} \left[ (b_1 + a_2) \sqrt{\sqrt{G'^2 + G''^2} + G'} + (a_2 - b_1) \sqrt{\sqrt{G'^2 + G''^2} - G'} \right] \quad (2)$$

$$g_1 = \frac{\pi}{4} \rho b^2 \omega \left( \frac{\frac{(m_c + m_A)\omega}{Q} - c_i}{\rho b^2 L \omega \frac{\pi}{4}} \right) \quad (3)$$

$$g_2 = \frac{\pi}{4} \rho b^2 \left( \frac{4\mu \left( \frac{\omega_0^2}{\omega^2} - 1 \right)}{\pi b^2 \rho} \right) \quad (4)$$

where  $L$  is the cantilever length,  $\mu = \rho_c b t$  is the cantilever mass per unit length,  $\rho_c$  and  $t$  are the respective cantilever density and thickness,  $Q_0$  and  $\omega_o$  are the respective quality factor and resonant frequency in the absence of fluid damping (i.e., resonating in vacuum with only internal damping effects present),  $m_c = \rho_c b t L$  is the cantilever mass,  $m_A = \rho \pi b^2 L \Gamma' / 4$  is the added mass,  $\Gamma'$  is the real part of the hydrodynamic function, and  $c_i = m_c \omega_o / Q_o$  is the internal damping coefficient. Due to

the scale of the cantilevers ( $L = 4.1\text{ mm}$ ) the internal damping was not negligible and was subtracted from the measured value (as described in the term  $c_i$  in Equation 3). In calculation of  $c_i$ ,  $\omega_o$  and  $Q_0$  were approximated as  $\omega_o \sim 2\pi f_{n,air}$  and  $Q_0 \sim Q_{n,air}$ , which were reasonable assumptions as discussed in the following sections. The hydrodynamic function was approximated using the relation  $\Gamma' = a_1 + a_2\delta/b$ , where  $\delta = [(2\eta/(\rho\omega))]^{1/2}$  is the thickness of the thin viscous layer surrounding the cantilever in which the velocity has dropped by a factor of  $1/e$ , and  $\eta$  is the viscosity of the fluid. Given the significant change in resonance amplitude associated with gelation, this study focused on characterization of ICE hydrogel gelation based on real-time monitoring of  $\phi$ ,  $G'$ , and  $G''$ . In Figures 3.2G-I,  $G'$  and  $G''$  at  $f$  increased throughout the gelation process, similar to the trend of low-frequency viscoelastic moduli.<sup>125</sup>

Having established the measurement principle and format associated with PEMC sensor-based real-time monitoring of composite PNIPAM-alginate hydrogel viscoelastic properties, we next examined the effect of hydrogel composition on the sensor response and associated viscoelastic properties. A comparison of the change in resonance amplitude (i.e., peak height =  $\phi - \phi_0$ ) associated with PNIPAM and alginate network formation during composite gelation is shown in Figure 3.3A using PNIPAM and alginate as negative controls (i.e. gelation in the absence of the other species). We found that both the change in resonance amplitude and viscoelastic properties caused by both PNIPAM and alginate network formation was lower in the composite PNIPAM-alginate hydrogel than in pure control hydrogels. For example, photocuring of NIPAM solution in the absence of alginate resulted in a  $19.2 \pm 1.3\%$  decrease of the resonance amplitude, while photocuring of NIPAM solution that contained alginate, which we refer to as the composite hydrogel precursor solution, decreased to a lesser extent ( $15.55 \pm 1.05\%$ ). Similarly, chemical crosslinking of alginate solution in the absence of PNIPAM caused the resonance amplitude to

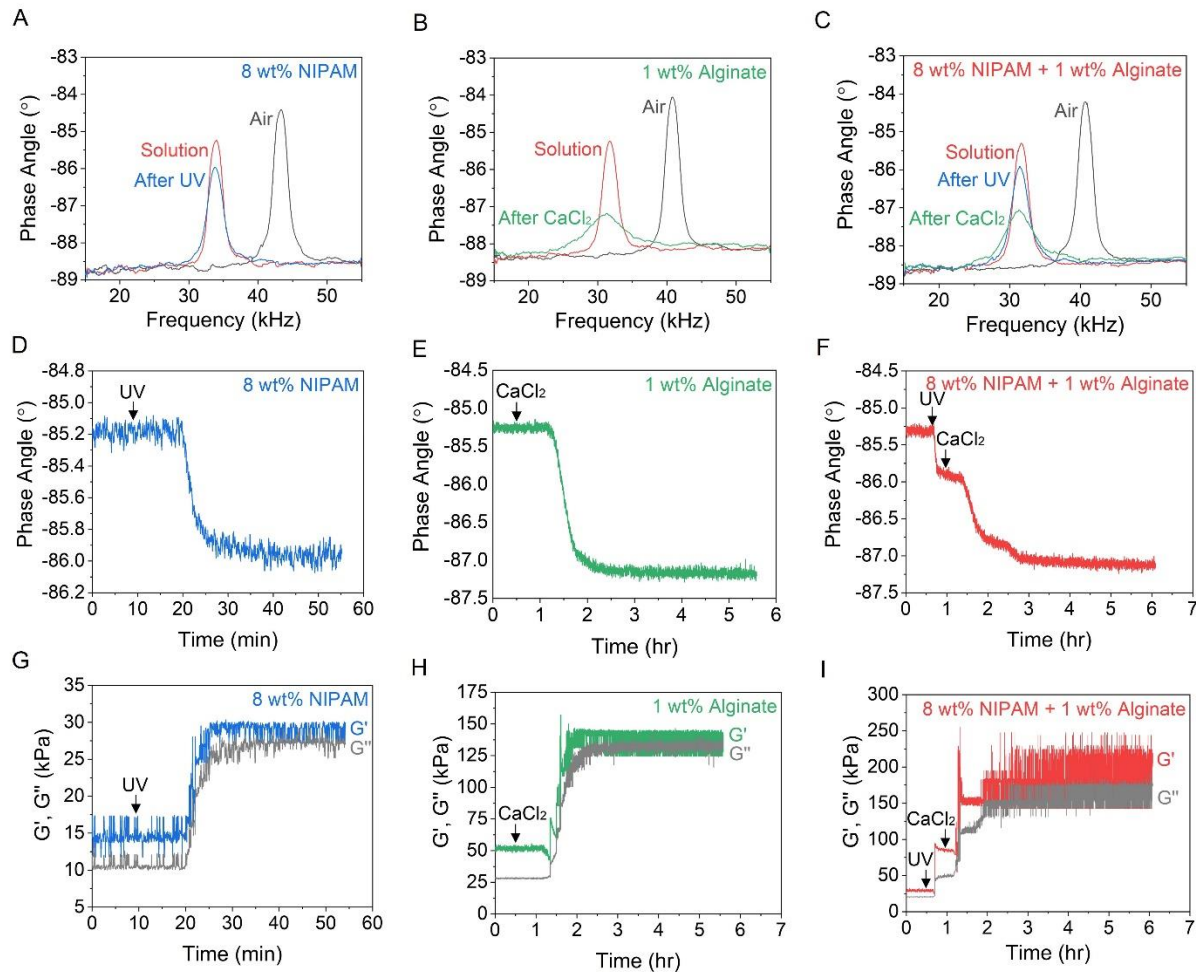


**Figure 3.2** PEMC sensor impedance spectra (phase shift) in air, solution, and hydrogel for PNIPAM (A), alginate (B), and composite PNIPAM-alginate (C) precursor solutions and hydrogels. Real-time monitoring of PNIPAM, alginate, and composite PNIPAM-alginate hydrogel gelation via continuous tracking of phase angle at resonance ( $\phi$ ) (D, E, F) and associated real-time change in high-frequency shear moduli ( $G'$ ,  $G''$ ) at the resonant frequency ( $f$ ) (G, H, I).

decrease by  $52.7 \pm 3.5\%$ , while chemical crosslinking of the composite PNIPAM-alginate hydrogel caused a decrease of  $41.1 \pm 1.4\%$ . This result suggests that the presence of the additional macromolecular component potentially affected the network structure relative to that achieved in hydrogels that involve a single macromolecular component (e.g., pure PNIPAM and alginate

hydrogels). This is a reasonable observation given the remaining free volume within the network in the case of the composite systems, which can be occupied by an interpenetrating network. The total decrease in the resonance amplitude caused by composite PNIPAM-alginate hydrogel gelation ( $50.2 \pm 1.3\%$ ) was comparable to that achieved by pure alginate hydrogels ( $52.7 \pm 3.5\%$ ). A comparison of shifts in  $f$  and  $Q$  caused by gelation are provided in Supporting Figure S3.

In addition to the change (decrease) of resonance amplitude, real-time monitoring of  $\phi$  also provided insight into dynamic processes, such as the reaction kinetics (e.g., photopolymerization reaction) and mass transfer processes (e.g., monomer or crosslinker diffusion), that drive the gelation process. A comparison of the data shown in Figures 3.2D - I shows that alginate gelation was slower in the presence of a crosslinked PNIPAM network compared to the absence of PNIPAM (i.e., for pure alginate hydrogels). This observation is consistent with the decreased diffusivity of  $\text{Ca}^{2+}$  ions through a polymer network relative to solution. Thus, these results suggest that PEMC sensors provide a useful analytical platform for investigating the dynamic processes that drive hydrogel property response to external stimuli.

It is well established that  $G'$  scales as  $\omega^{0.5}$  for many crosslinked polymers near the gel point, such as biomaterials.<sup>126-128</sup> In the gel phase, the increase in  $G'(\omega)$  at high frequencies is because the bond lifetime is now longer than the typical motion due to thermal fluctuations; thus, the system shows the typical increase in elasticity, as the system cannot relax fast enough.<sup>127</sup> However, while this is a general scaling law for biomaterials, previous research has shown that  $G'$  may also exhibit power-law relations across the  $1\text{-}10^4$  rad/s angular frequency range.<sup>127</sup> A comparison of  $G'$  and  $G''$  at 1 Hz and  $f$  ( $\sim 30$  kHz) obtained by DMA and the PEMC sensor, respectively, among the different hydrogels is shown in Figure 3.3B. The  $G'$  at  $f$  was larger than the  $G'$  at 1 Hz by greater than one order of magnitude among all hydrogels, ranging from a factor of  $\sim 10$  - 30. These observations are

not unreasonable when we consider the magnitude of  $f$  compared to the inverse of the surrounding material relaxation time, which is relatively slow.<sup>129</sup> The ratio of  $G'$  at  $f$  to  $G'$  at 1 Hz obtained by DMA and sensor data in this study agree reasonably with microrheology studies, which reported  $G'(2\pi f = 30 \text{ kHz})/G'(2\pi f = 1 \text{ Hz})$  ranged from  $\sim 10 - 100$  for water and DNA hydrogels across the  $20 - 50^\circ \text{C}$  temperature range.<sup>127</sup> The  $G'$  obtained by sensor data were  $42.1 \pm 18.9$ ,  $159.8 \pm 24.2$ , and  $188.4 \pm 34.7 \text{ kPa}$  for PNIPAM, alginate, and composite PNIPAM-alginate hydrogels, respectively. The corresponding  $G''$  were  $32.2 \pm 10.8$ ,  $145.9 \pm 33.5$ , and  $160.2 \pm 34.8 \text{ kPa}$  for PNIPAM, alginate, and composite PNIPAM-alginate hydrogels, respectively. In comparison, the  $G'$  obtained by DMA were  $1.2 \pm 0.2$ ,  $11.8 \pm 4.3$ , and  $17.3 \pm 4.1 \text{ kPa}$  for PNIPAM, alginate, and composite PNIPAM-alginate hydrogels, respectively. The corresponding  $G''$  were  $0.06 \pm 0.01$ ,  $2.2 \pm 0.9$ , and  $1.5 \pm 0.3 \text{ kPa}$  for PNIPAM, alginate, and composite PNIPAM-alginate hydrogels, respectively. Importantly,  $G'$  and  $G''$  at  $f$  obtained by sensing are correlated with  $G'$  and  $G''$  obtained by DMA for composite PNIPAM-alginate hydrogels. In addition to understanding the dependency of  $G'$  on  $f$  (i.e.,  $\omega$ ), we next compared results obtained by DMA and PEMC by examining the effect of the frequency on  $\delta$  and complex shear modulus ( $|G^*|$ ). High-frequency rheology data obtained by MEMS-based techniques, such as QCM-based methods, have also been validated previously by comparison with low-frequency rheology data obtained by dynamic mechanical analysis.<sup>130</sup> In that study, the resonant frequency changes associated with the first three odd resonant modes were utilized to characterize the high-frequency viscoelastic properties. Validation of QCM data against DMA data was previously done by comparison of  $\delta$  vs.  $|G^*|$  obtained under the same conditions. Here, we found that  $\delta$  and  $G^*$  at 1 Hz obtained by DMA for the alginate, PNIPAM, and alginate-PNIPAM hydrogels were  $2.9$ ,  $10.6$ , and  $5.0^\circ$  and  $1.2$ ,  $12.0$ , and  $17.4 \text{ kPa}$ , respectively;  $\delta$  and  $G^*$  at  $\sim 30 \text{ kHz}$  obtained by PEMC for the alginate, PNIPAM, and

alginate-PNIPAM hydrogels were 37.4, 42.4, and 40.4° and 53.0, 216.4, and 247.3 kPa, respectively. Thus,  $\delta$  obtained at high-frequency were relatively increased than that observed at low frequency by DMA for similar  $G^*$  across the materials investigated. This result is consistent with previous QCM-based studies, which showed that the viscoelastic phase lag of the third mode of QCM near 15 MHz was relatively increased in polybutadiene and styrene-butadiene compared with that observed by DMA.<sup>130</sup> Differences in the change of  $\delta$  between low- and high-frequency in this study and the aforementioned QCM-based study are attributed to differences in the range of the complex modulus investigated (kPa vs. MPa – GPa, respectively) and mode type used for rheological characterization (second-order bending mode near 30 kHz vs. third-order shear mode near 15 MHz, respectively). While PEMC and QCM provide sensor-based characterization of high-frequency viscoelastic properties that are consistent with DMA, PEMC sensors exhibit several advantages, particularly associated with the sensor form factor. One significant challenge in the materials science and engineering is the ability to characterize small sample volumes. Similar to QCM, which exhibits surface area  $\sim 1 \text{ cm}^2$ , PEMC sensors also facilitate characterization of droplets by deposition on the top face of the cantilever ( $\sim 1 \text{ mm}^2$ ). However, the millimeter-scale dip-stick-based form factor offers unique advantages over planar centimeter-scale QCM geometry in terms of accessibility to small sample containers through vertical submersion. For example, PEMC sensors are 1 mm in width, which enables submersion in a variety of plastic- and culture-ware, including microcentrifuge tubes and 96-well plates, which are commonly utilized in materials science and additive manufacturing applications. Thus, we next examined if the dynamic response of composite hydrogel viscoelastic properties could be continuously monitored during material stimulation to further support their use in future process monitoring applications.

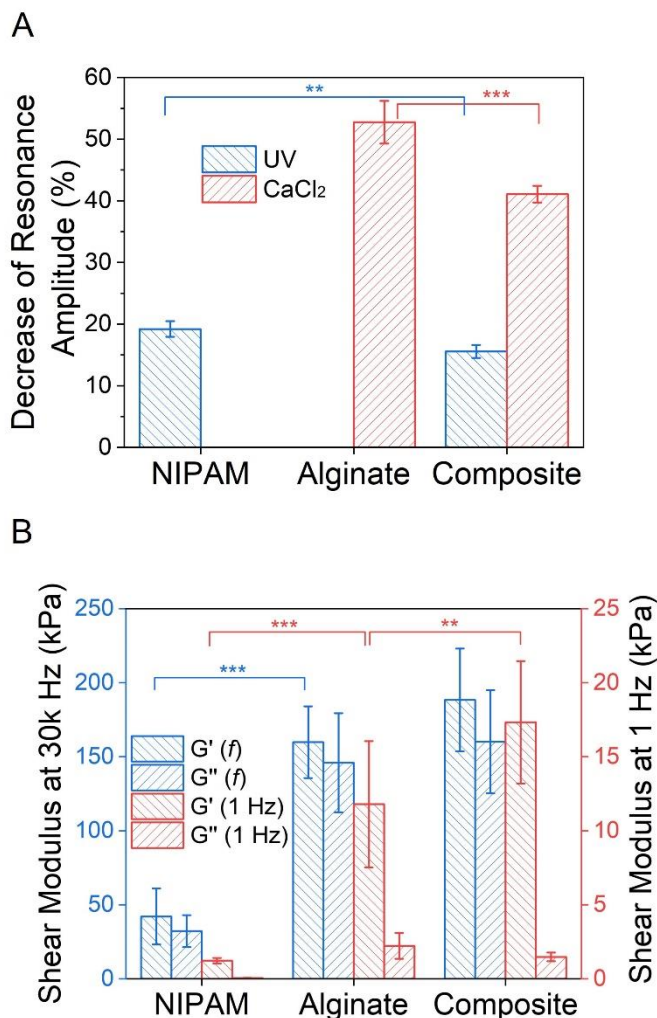


**Figure 3.3** (A) Comparison of the resonance amplitude change (i.e., peak height change) at each step of gelation process. (B) Comparison of shear moduli ( $G'$ ,  $G''$ ) at the resonant frequency ( $f$ ) obtained by sensing with  $G'$  and  $G''$  at 1 Hz obtained by DMA assuming Poisson ratio ( $\nu$ ) = 0.5 for PNIPAM, alginate, and composite PNIPAM-alginate hydrogels (\*, \*\*, and \*\*\* indicate  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively).

### 3.3.2 Real-time Sensor-based Monitoring of ICE Composite PNIPAM-Alginate Hydrogel Thermal Response

At present, while image-based methods facilitate real-time monitoring of stimuli-responsive hydrogel and LBL-cured construct geometry change under various stimuli, few methods enable

real-time monitoring of construct property response to stimuli, such as dynamic response of viscoelastic properties. Thus, we next characterized the real-time viscoelastic property response of composite PNIPAM-alginate hydrogel constructs to thermal stimulation. Sensor-embedded PNIPAM, alginate, and composite PNIPAM-alginate hydrogels were heated from room temperature (25 °C) to body temperature (37 °C) and then cooled to 25 °C. The range was selected based on the potential biomedical applications. Figures 3.4A-C show the effect of temperature on the sensor resonance amplitude when surrounded by the various hydrogels. The continuous  $\phi$  responses to the temperature cycling (i.e., raw sensor time-series data) are shown in shown in Figures 3.4D-F. The  $f$  and  $Q$  responses are provided in Supporting Figure SA - 4. The sensor responses exhibited profiles associated with the conductive heating and cooling processes used for temperature control. The PNIPAM, alginate, and composite PNIPAM-alginate hydrogels stabilized at  $16.6 \pm 3.6$ ,  $18.1 \pm 1.7$ , and  $16.9 \pm 1.0$  min following the temperature step change from 25 to 37 °C, respectively. The dynamic response of the composite PNIPAM-alginate viscoelastic properties vs. the control hydrogel responses based on the real-time sensor data and a fluid-structure interaction model are shown in Figures 3.4G-I.<sup>114, 115</sup> The effect of temperature change on the hydrogel viscoelastic properties was significantly higher in PNIPAM and composite PNIPAM-alginate hydrogels relative to alginate hydrogels. The viscoelastic property response of PNIPAM to temperature cycling also exhibited two maxima of  $G'$  at  $f$  during the heating and cooling phases (see Figure 3.4G). A similar trend in  $G'$  was also present for the composite PNIPAM-alginate hydrogel (see Figure 3.4I). We note that this trend was not observable in the alginate hydrogel response (Figure 3.4H), suggesting that it may be associated with thermal response of the PNIPAM network in the pure and composite hydrogel systems.

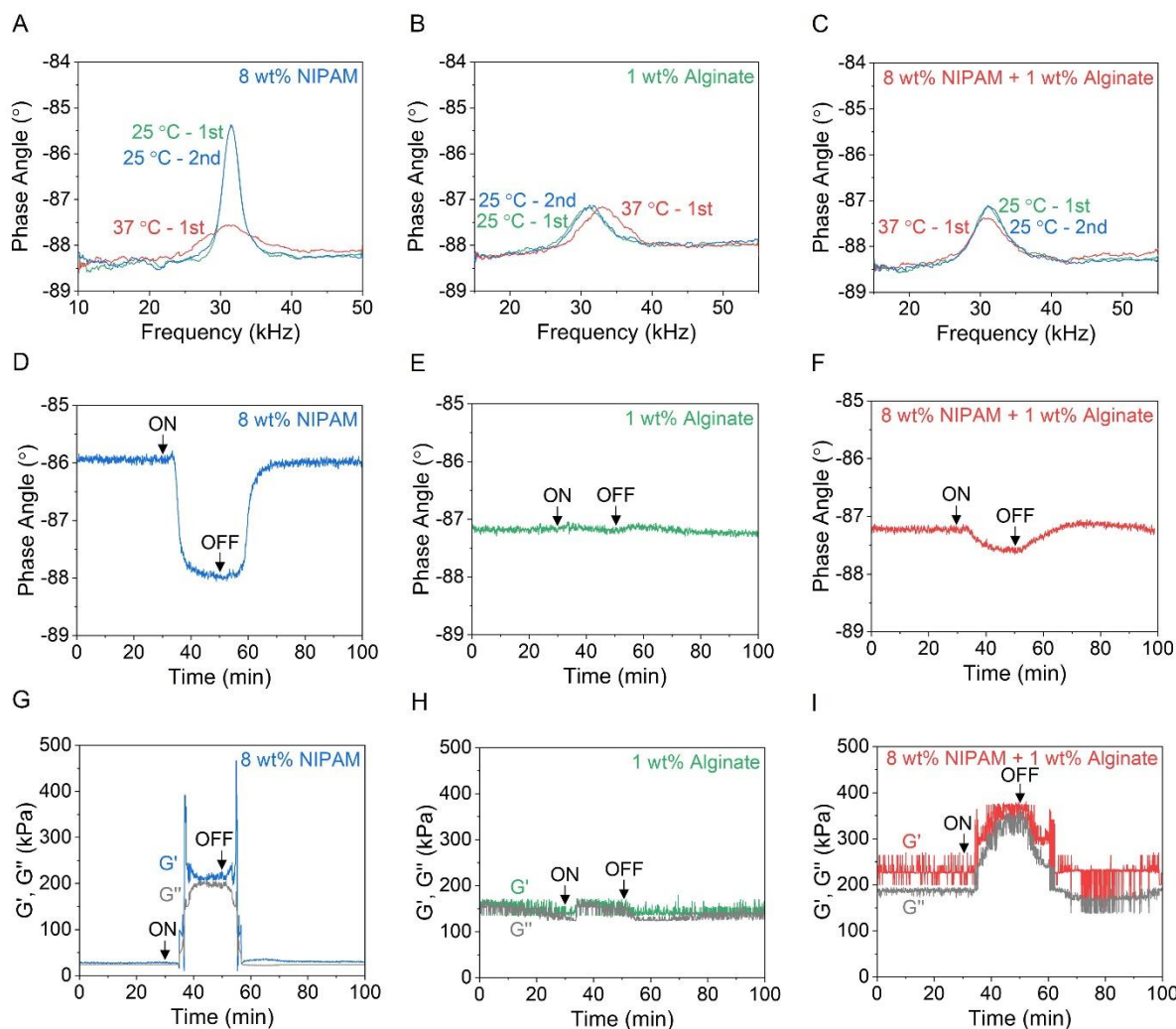


**Figure 3.4** PEMC sensor impedance spectra (phase shift) in NIPAM (A), alginate (B), and NIPAM-alginate (C) hydrogels at  $T = 25$  and  $37$  °C, and after one heating-cooling cycle. Real-time monitoring of phase angle ( $\phi$ ) (D, E, F) and shear moduli ( $G'$  and  $G''$ ) at the resonant frequency ( $f$ ) (G, H, I) throughout a heating-cooling cycle of PNIPAM, alginate, and composite PNIPAM-alginate composite hydrogel.

As shown in Figure 3.5A, the extent of change in the percentage of peak amplitude (i.e.,  $\phi$ ) was dependent on the hydrogel type. PNIPAM exhibited the most extensive relative thermal response based on  $\phi$  change, which decreased by  $67.3 \pm 4.4\%$  upon heating from  $25$  to  $37$  °C. The

composite PNIPAM-alginate hydrogels exhibited the second largest response to the thermal stimuli, which caused a decrease of the resonance amplitude by  $20.7 \pm 2.7\%$ . The alginate hydrogel exhibited the smallest response to thermal stimuli ( $2.4 \pm 4.7\%$  decrease of resonance amplitude). This trend agrees with previously reported temperature response data.<sup>131, 132</sup> For example, Safakas et al. found the thermo-induced response of alginate-g-PNIPAM hydrogels shift according to the content of NIPAM. It is also well established that PNIPAM hydrogels exhibit relatively large changes in shape and properties upon temperature change, which is not the case for alginate. Thus, the composite PNIPAM-alginate hydrogel exhibited dynamic viscoelastic property changes that were intermediate of the single component hydrogels.

Figures 3.5B provides a comparison of the temperature dependence of the viscoelastic properties of PNIPAM, alginate, and composite PNIPAM-alginate hydrogels. The  $G'$  at 25 °C was  $42.1 \pm 18.9$ ,  $159.8 \pm 24.2$ ,  $201.6 \pm 24.0$  kPa for PNIPAM, alginate, and composite PNIPAM-alginate, respectively. The temperature increase to 37 °C caused a significant increase in  $G'$  of PNIPAM to  $241.1 \pm 57.2$ . In contrast, the temperature increase caused a small decrease in  $G'$  of alginate ( $146.7 \pm 5.2$  kPa). Similar to the PNIPAM hydrogel, the temperature increase from 25 to 37 °C was associated with an increase in  $G'$  of the composite PNIPAM-alginate hydrogel ( $290.5 \pm 5.5$  kPa). The sensor responses for PNIPAM hydrogels subjected to multiple temperature cycles are provided in Supporting Figure SA - 5. Thus, the observed changes are consistent with established knowledge of alginate and PNIPAM hydrogels, which further validated the use of PEMC sensor for real-time monitoring of stimuli-responsive hydrogel structure and properties.

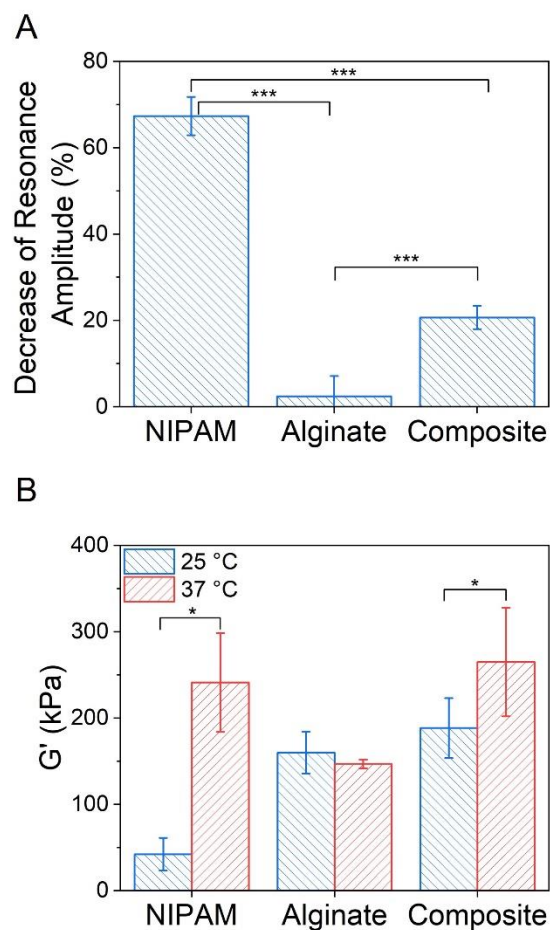


**Figure 3.5** (A) Decrease of resonance amplitude (i.e., difference between phase angle  $\phi$  at resonance and baseline value) after heating of PNIPAM, alginate, and composite PNIPAM-alginate hydrogels from 25 to 37 °C. (B) Storage modulus ( $G'$ ) at the resonant frequency ( $f$ ) of PNIPAM, alginate, and composite PNIPAM-alginate hydrogels at 25 and 37 °C (\*, \*\*, and \*\*\* indicate  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively).

As shown in Figure 3.6, the network structure of the composite PNIPAM-alginate hydrogels was significantly different below and above the VPTT ( $\sim 32$  °C), which supports the sensor data. PNIPAM hydrogels also exhibited significantly different network structures below and above the VPTT of PNIPAM ( $\sim 32$  °C; see Figure SA - 6). Specifically, these SEM images capture the

transition of the PNIPAM network structure from an expanded, open state with pores much larger than 100  $\mu\text{m}$  to a tighter, collapsed network with pores on the order of 10  $\mu\text{m}$ . The PNIPAM-alginate hydrogel constructs exhibited similar mesh sizes to neat PNIPAM hydrogels, which exhibited pore sizes on the order of hundreds of micrometers, below the VPTT of PNIPAM (see Figure SA - 7).<sup>133</sup> Note, no pores smaller than those on the order of tens of micrometers were observed at higher magnification.

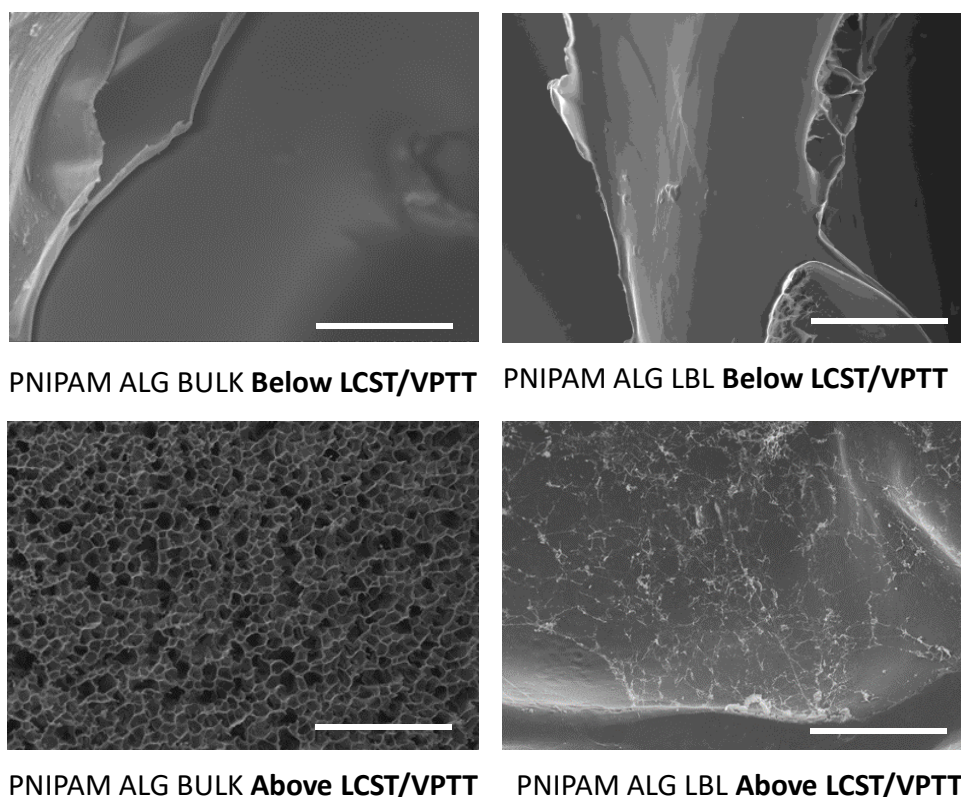


**Figure 3.6** SEM of bulk- (A, C) and layer-by-layer (LBL)-cured (B, D) composite PNIPAM-alginate hydrogels below (A, B) and above (C, D) the VPTT of PNIPAM (scale bar = 100  $\mu\text{m}$ ).

A similar transition in the network structure of the bulk-cured composite PNIPAM-alginate hydrogels was observed, where a tighter, more well-defined network structure, with pore sizes on the order of tens of micrometers, is observed for these materials once the samples were subjected

to temperatures above that of the VPTT of PNIPAM (Figure 3.6A vs. Figure 3.6C). The changes in the network structure captured by these SEM images helps to elucidate the mechanism behind the enhancement in the storage moduli shown in Table 1, where, for example, the storage modulus of the bulk-cured composite PNIPAM-alginate hydrogel increased from  $201.6 \pm 24.0$  kPa to  $290.54 \pm 54.6$  kPa, below and above the VPTT, respectively. However, the pore structure of these soft composites was more uniform, with smaller pore sizes, than that observed for the neat PNIPAM samples (Figure 3.6C vs. Figure SA - 6B).

**Table 3-1** Storage modulus ( $G'$ ) at the resonant frequency ( $f \sim 30$  kHz) of PNIPAM hydrogels, alginate hydrogels, bulk-cured composite PNIPAM-alginate hydrogels, layer-by-layer (LBL)-cured composite PNIPAM-alginate constructs at 25 and 37 °C calculated from sensor data and cantilever fluid-structure interaction theory.

| Hydrogel                   | $G'$ -25 °C<br>(kPa) | $G'$ -37 °C<br>(kPa) |
|----------------------------|----------------------|----------------------|
| PNIPAM                     | $42.1 \pm 18.9$      | $241.1 \pm 57.2$     |
| Alginate                   | $159.8 \pm 24.2$     | $146.7 \pm 5.2$      |
| Bulk-cured PNIPAM-alginate | $201.6 \pm 24.0$     | $290.5 \pm 54.5$     |
| LBL-cured PNIPAM-alginate  | $246.6 \pm 67.7$     | $408.2 \pm 110.9$    |

No well-defined network structure was observed above the VPTT, where only an apparent variation in the surface morphology was observed (Figure 3.6B vs. Figure 3.6D). In fact, above the VPTT of PNIPAM, the soft composite appeared to phase separate, creating a PNIPAM-rich phase with a web-like network structure and an alginate-rich phase with no apparent pores. The formation of this web-like structure by the PNIPAM provides physical insight into the mechanism of increased storage modulus and dynamic viscoelastic property response. Further, the fact that the

PNIPAM phase separated from the alginate may help to explain the larger increase in storage modulus below and above the VPTT for LBL-cured composite PNIPAM-alginate hydrogel ( $246.6 \pm 67.6.0$  kPa to  $408.2 \pm 110.9$  kPa) when compared to bulk-cured composite PNIPAM-alginate hydrogel ( $201.6 \pm 24.0$  kPa to  $290.54 \pm 54.6$  kPa), where the PNIPAM is prevented from phase separating due to the fabrication method for these hydrogels.

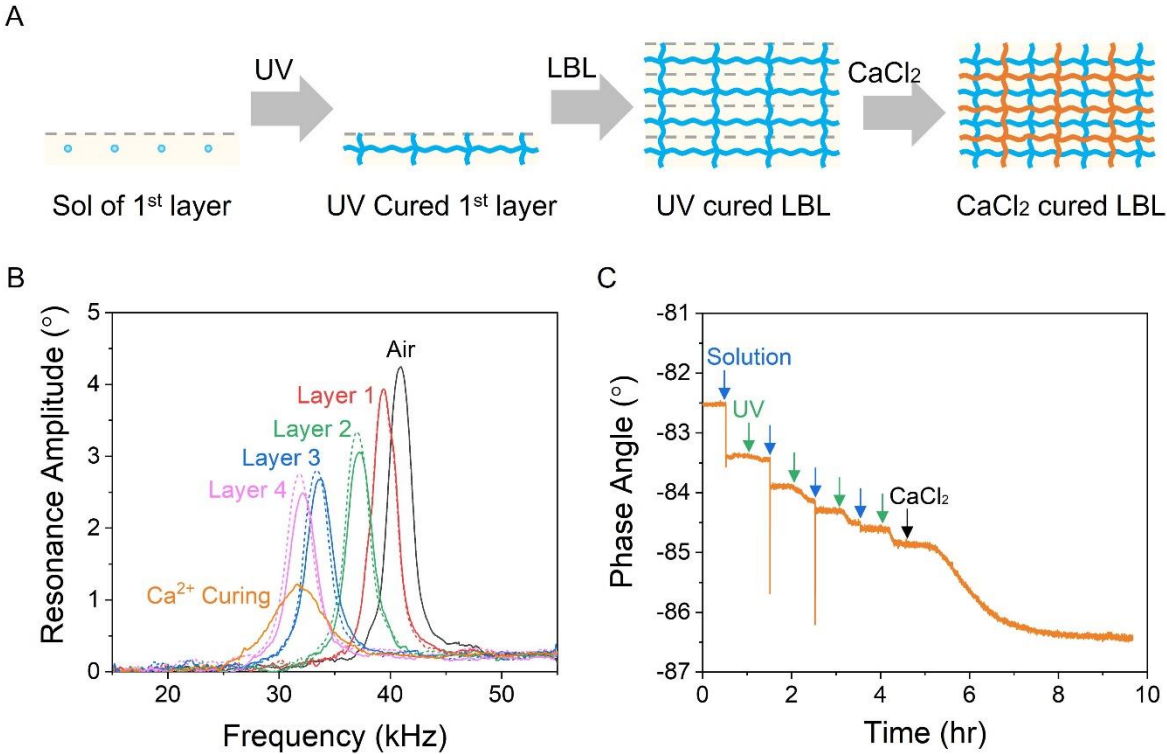


**Figure 3.7** (A) Schematic illustrating the LBL fabrication process (deposition and curing) associated with the LBL-cured composite PNIPAM-alginate constructs. (B) Phase angle spectra from the cantilever sensor obtained at various times throughout the gelation process (dotted and solid lines indicate the sol and UV-cured gel state of each layer, respectively). (C) Real-time monitoring of LBL composite PNIPAM-alginate construct fabrication via continuous tracking of cantilever  $\phi$  response.

### 3.3.3 Real-time Monitoring of Composite PNIPAM-Alginate LBL Construct Gelation and Comparison of Bulk- vs. LBL-cured Construct Dynamic Viscoelastic Property Response

Stimuli-responsive hydrogels have great potential applications in additive manufacturing based on their ability to change shape and properties upon stimulation after fabrication. While a considerable number of studies have been reported on additively manufactured constructs, which utilize LBL deposition and curing processes, few studies and methods have characterized the real-time material assembly or dynamic response of bulk vs. LBL-cured PNIPAM-alginate constructs. Thus, having monitored the real-time response of bulk-cured composite PNIPAM-alginate hydrogels to thermal stimuli, we next examined the assembly (gelation) and stimuli response of LBL-cured composite PNIPAM-alginate constructs to understand the potential impact of the fabrication (i.e., deposition and curing) approach (bulk- vs. LBL-cured) on the network structure and dynamic viscoelastic response to thermal stimuli. An illustration of the LBL composite PNIPAM-alginate construct fabrication, which resembles an additive manufacturing process, is shown in Figure 3.7A. The average layer thickness was 0.6 mm based on the commonly used layer spacing in microextrusion 3D printing applications. Figure 3.7B highlights the effect of layer curing of the impedance spectra of the PEMC sensor. Deposition of the polymer solution around the sensor caused a significant shift in both  $f$  and  $\phi$ . Subsequent photocuring of each layer resulted in a continuous change of  $\phi$  as observed previously during fabrication of bulk-cured composite PNIPAM-alginate hydrogels (see Figure 3.2). A significant decrease in  $\phi$  was observed upon final chemical crosslinking of the four-layer LBL-photocured composite PNIPAM-alginate construct with  $\text{CaCl}_2$ . Figure 3.7C shows the real-time  $\phi$  response during the LBL composite PNIPAM-alginate construct gelation process, which was composed of four subsequently photocured layers followed by a final chemical crosslinking of the LBL-cured construct. The real-time sensor response associated with the LBL processing is shown in Supporting Figure SA - 8.

Having monitored the fabrication of LBL-cured composite PNIPAM-alginate constructs, we next compared their gelation and stimuli response with the previously examined bulk-cured samples. As shown in Figure 3.8A, the curing method had a significant effect on the change in  $\phi$  (i.e., the expected change in  $G'$  associated with crosslinking). For bulk-cured composite PNIPAM-alginate hydrogels, the changes of  $\phi$  caused by UV curing and  $\text{CaCl}_2$  crosslinking were  $36.0 \pm 3.3\%$  and  $41.1 \pm 1.4\%$ , respectively. However, for LBL-cured composite PNIPAM-alginate constructs the changes of  $\phi$  associated with UV curing,  $\text{CaCl}_2$  crosslinking, and the total change were  $39.1 \pm 3.5\%$ ,  $46.4 \pm 3.5\%$ , and  $67.3 \pm 3.6\%$ , respectively.

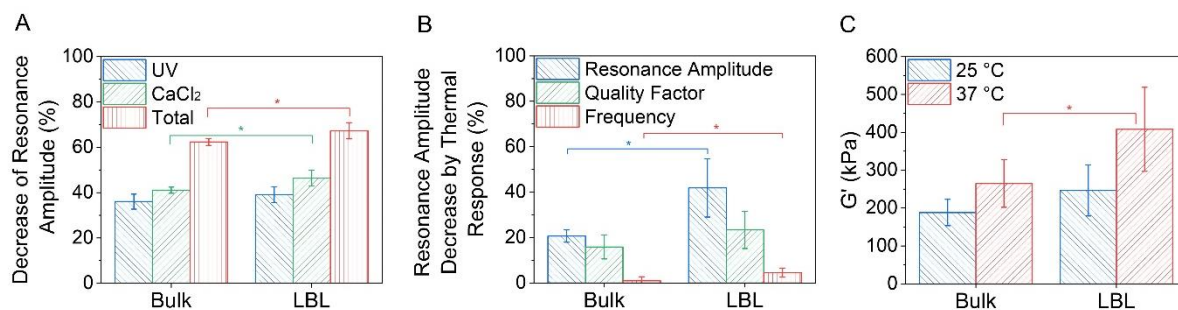


**Figure 3.8** (A) Comparison of the resonance amplitude decrease caused by gelation for bulk and layer-by-layer (LBL) composite PNIPAM-alginate hydrogels (i.e., bulk- vs. LBL-cured constructs). (B) Comparison of the percent decrease in resonance amplitude (peak height) caused by the thermal response of bulk and LBL composite PNIPAM-alginate hydrogels in terms of three sensor outputs from  $T = 25$  and  $37$  °C. (C) Comparison of the storage modulus of bulk and LBL composite PNIPAM-alginate at  $T = 25$  and  $37$  °C.

We then compared the real-time thermal response of bulk- and LBL-cured hydrogels via continuous monitoring of  $\phi$  response. Figure 3.8B shows the percent change of  $\phi$  upon heating the composite PNIPAM-alginate hydrogels from  $25$  to  $37$  °C, indicating  $20.7 \pm 2.7\%$  and  $41.9 \pm 12.8\%$  change for the bulk and LBL-cured composite hydrogels respectively ( $p = 0.022$ ). Similarly, the

bulk- and LBL-cured composite hydrogels exhibited significantly different percent changes in  $f$  from heating of  $1.0 \pm 1.8\%$  and  $4.6 \pm 2.0\%$  ( $p = 0.016$ ). Next, we compared  $G'$  of bulk- and LBL-cured composite PNIPAM-alginate constructs at 25 and 37 °C (see Figure 3.8C). LBL-cured composite PNIPAM-alginate constructs exhibited a significantly larger  $G'$  change compared to bulk-cured constructs ( $p = 0.04$ ). Thus, these studies suggest that the magnitude of dynamic viscoelastic property response of composite PNIPAM-alginate constructs is dependent on processing method (e.g., material deposition-assembly and curing processes).

### 3.4 Conclusions

In this work, dynamic-mode piezoelectric milli-cantilever sensors were utilized to monitor the real-time gelation and thermal response of ionic-covalent entanglement composite PNIPAM-alginate hydrogel, a widely investigated stimuli-responsive hydrogel system. The dynamic response of composite PNIPAM-alginate viscoelastic properties during network formation and thermal stimulation were monitored in real-time based on sensor data and established fluid-structure interaction models. Trends in shear moduli at the resonant frequency obtained by sensing correlated with compressive moduli data obtained by DMA. SEM captured the transition of the network structure of PNIPAM–alginate composite hydrogels below and above the VPTT of PNIPAM, which was quantitatively supported by changes in values of storage modulus with temperature. We also showed that the magnitude of dynamic viscoelastic property response in composite PNIPAM-alginate hydrogels is dependent on the fabrication method based on the comparison of bulk- and layer-by-layer constructs. In summary, piezoelectric milli-cantilever sensors are a useful experimental tool for characterization and real-time monitoring of hydrogel response to stimuli in practical low-volume measurement formats.

## **4 Accelerated Engineering of Optimized Functional Composite Hydrogels via High-Throughput Experimentation**

The Materials Genome Initiative (MGI) seeks to accelerate the discovery and engineering of advanced materials via high-throughput experimentation (HTE), which is a challenging task given the common tradeoff between design for optimal processability vs. performance. Here, we report a HTE method based on automated formulation, synthesis, and multi-property characterization of bulk soft materials in well plate formats that enables accelerated engineering of functional composite hydrogels with optimized properties for processability and performance. The method facilitates rapid high-throughput screening of hydrogel composition-property relations for multiple properties in well plate formats. The feasibility and utility of the method were demonstrated by application to several functional composite hydrogel systems, including alginate/poly(*N*-isopropylacrylamide) (PNIPAM) and poly(ethylene glycol) dimethacrylate (PEGDMA)/poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS) hydrogels. The HTE method was leveraged to identify formulations of conductive PEGDMA/PEDOT:PSS composite hydrogels for optimized performance and processability in 3D printing. This work provides an advance in experimental methods based on automated dispensing, mixing, and sensing for accelerated engineering of soft functional materials.

### **4.1 Introduction**

The Materials Genome Initiative (MGI) aims at accelerating the pace of materials discovery, engineering (e.g., optimization), and commercialization while minimizing resource expenditures through innovation in materials research infrastructure. Creating new experimental tools and methods for rapid experimentation, which includes formulation, synthesis, and characterization, remains a critical bottleneck. High-throughput experimentation (HTE) is foundational to

accelerating the pace and impact of simulation, such as through model validation and support of digital twin, and also provides a direct path to experimentation-based accelerated materials discovery in the absence of simulation.<sup>3</sup> HTE is now central to the pharmaceutical industry and other industries and fields based on molecular engineering and technology and is presently evolving to support accelerated materials discovery and engineering.<sup>134</sup> For example, HTE for thin film materials research, driven by the translatability of existing processes and analytical techniques for thin film synthesis and characterization, has received considerable attention to date. Associated HTE methods for thin film systems have been applied to a range of applications, such as those associated with electronic, magnetic, optical, and energy-related materials.<sup>3, 9</sup>

Compared with hard materials, relatively less progress has been made in HTE of soft materials (e.g., hydrogels) due to challenges associated with adapting conventional methods for formulation, synthesis, and characterization of soft materials to high-throughput formats. However, stemming from progress in rapid prototyping (e.g., via additive manufacturing), new methods for high-throughput formulation and synthesis of soft materials are now emerging, such as for high-throughput synthesis (HTS) and high-throughput characterization (HTC) of hydrogels. Microfluidics now serve as a common HTE format for accelerated hydrogel engineering. For example, Kumachev *et al.* recently reported a microfluidic approach for HTS of hydrogel microbeads with varying elasticity for cell encapsulation.<sup>71</sup> While microfluidic-based formats exhibit several attractive characteristics, such as continuous production modality, they are relatively limited regarding ease of interface with HTC (i.e., the characterization of materials generated via microfluidic-based HTE formats presents a bottleneck).<sup>69, 70</sup> Robotic dispensing, commonly in microarray geometry, also provides another HTE format for accelerated hydrogel engineering. In this method, hydrogel precursor solutions are typically deposited on a substrate

in a drop-wise manner, such as via inkjet printing, enabling the formulation of a ‘library’ of hydrogel samples via spatial control of the deposited volume of each component (e.g., precursor solution or crosslinking agent).<sup>61, 62, 64, 72</sup> Fabrication of functionally graded materials has also emerged as a method for accelerated engineering of hydrogels,<sup>74, 135</sup> but is relatively constrained regarding interface with HTC methods as the method does not produce a large number of samples. While several useful HTE formats for accelerated engineering are emerging, quality assurance and quality control remain critical areas, such as verification of component mixing. In addition, formats based on microfluidics and microarray typically generate microscale samples, which may complicate the translation of generated process-composition-structure-property relations to the design of macroscale constructs (e.g., soft robotics) and present challenges associated with benchmarking with established characterization data or results obtained from simulation of bulk material behavior. Toward addressing these challenges, integrated robotic dispensing and molding processes have served as an attractive format for accelerated hydrogels engineering.<sup>65, 77</sup> Compared with microfluidic, microarray, and graded materials-based methods, the format generates large quantities of bulk samples in substrates (e.g., well plates) that synergize with a range of HTC methods. However, it remains a challenge to execute HTC of disparate properties from large batches of synthesized samples with varying composition (i.e., hydrogel libraries), such as physical or mechanical properties, that are essential for assessing and optimizing both processability and performance.

HTC methods can be broadly characterized as based on microfluidics, arrays, or robotics.<sup>61, 62, 65, 69, 70</sup> While several HTC methods exist for hard thin materials, often based on adapted spectroscopic methods, fewer exist for HTC of bulk soft materials. In particular, HTC of bulk soft material mechanical properties in conventional high-throughput formats, such as well plates,

remains a challenge.<sup>136</sup> As a result, characterization data for critical properties that inform hydrogel processability and performance are sometimes generated by selecting representative samples from the library, which has several limitations. The challenge originates from the limited throughput associated with conventional characterization methods for hydrogels (e.g., dynamic mechanical analysis) and the incompatibility of sample geometry among emerging HTS methods and established characterization methods. For example, microparticle geometries generated by microfluidic- and microarray-based formats are often incompatible with traditional rheological measurements.<sup>64, 71, 137</sup> Likewise, traditional characterization methods (e.g., atomic force microscopy) may require sample immobilization on a substrate.<sup>138</sup> Thus, optical-based HTC methods, such as based on fluorescence spectroscopy or image analysis, are commonly used to characterize large batches of hydrogel samples produced via HTS. Toward addressing these limitations, new HTC methods for hydrogels are continuing to emerge. For example, Andre *et al.* reported a new approach for characterizing the viscoelastic properties of droplets by shape analysis.<sup>139</sup> Oevreeide *et al.* described the potential to characterize microgel mechanics in microchannels.<sup>140</sup> Marquez *et al.* developed an indentation system for HTC of hydrogel tissue construct mechanics, while Xu *et al.* used a commercial indenter for HTC of hydrogel swelling characteristics and mechanical properties.<sup>65, 141</sup> Zhang *et al.* recently established a method for HTC of hydrogel rheological properties in 96-well plate formats via robotically-directed sensing using piezoelectric milli-cantilever sensors.<sup>116, 136, 142-144</sup> However, there remains a need for HTE formats capable of integrated high-throughput formulation, HTS, and HTC of complementary multi-property information that can support the accelerated engineering of functional composite hydrogels with optimized processability and performance, particularly in practical cross-disciplinary formats, such as well plates.

In our previous work, we established a HTC platform for characterization of solution and hydrogel rheological properties in well plate formats.<sup>136</sup> Here, we establish a HTE method based on automated sample preparation (i.e., dispensing, mixing, and synthesis) and multi-property characterization in standard 96-well plate formats. We focus on processing of hydrogel precursor solutions across a wide viscosity range. The HTE method is leveraged to identify formulations of several functional composite hydrogels that exhibit optimized processability and performance.

## 4.2 Experimental

### 4.2.1 Materials

Poly(ethylene glycol) dimethacrylate (PEGDMA,  $M_n = 750$ ), 2,2-dimethoxy-2-phenylacetophenone (DMPA), alginic acid sodium salt, calcium chloride ( $\text{CaCl}_2$ ), N-isopropylacrylamide (NIPAM), and N,N'-methylenebisacrylamide (MBAA), poly(3,4-ethylenedioxythiophene)-poly(styrene sulfonate) (PEDOT:PSS, 1.3wt%) were purchased from Sigma-Aldrich. Lead zirconate titanate (PZT-5A,  $72.4 \times 72.4 \times 0.127 \text{ mm}^3$ ) with nickel (Ni) electrodes was from Piezo Systems (Woburn, MA). Glass cylinders and ethanol (200 proof) were from Fisher Scientific. Polyurethane (Fast-Drying) and Loctite EA 1C-LV epoxy adhesive were from Minwax and Henkel, respectively. De-ionized water (DIW) ( $18.2 \text{ M}\Omega$ ) was from a DIW system (Direct-Q® 3 UV Water Purification Systems; Millipore).

### 4.2.2 Automated High-Throughput Formulation and Synthesis of Hydrogels via Robotically-directed Multi-material Dispensing and Mixing

Hydrogel stock precursor solutions were dispensed using a custom robot-assisted liquid dispensing system composed of a robot (F5200N; FISNAR) and dispensing system (Ultimus V; Nordson EFD). The viscosities of processed solutions were measured by a digital rotational viscometer (ViscoQC 100; Anton Paar) at room temperature. The precursor solutions were

dispensed from a syringe barrel (55 or 10 mL) using a straight cylindrical nozzle (32 or 27 gauge). For a given stock precursor solution, the mass of dispensed solution was controlled by the dispensing pressure, dispensing time, and size of the dispensing nozzle, which ranged from 1.9 – 27.3 kPa, 0 – 47.5 s, and 32 - 27 gauge, respectively. The dispensing parameters were selected to achieve a linear relationship between the mass of the dispensed solution and dispensing time per well prior to each experiment to ensure composition control for each sample (dispensing times for the different precursor solutions are described in the following sections). Here, the lower threshold was set as 0, and the upper threshold was set based on the dynamic range of the sensor (i.e., the concentration at which the cantilever quality factor drops below the threshold required for resonance tracking). We note that depending on the level of knowledge associated with the material system under investigation, the concentration ranges for individual components could be selected based on prior knowledge. In cases of accelerated materials discovery applications in which limited information may exist about the interaction among individual components or resultant material properties (e.g., phase behavior), several rounds of HTE may be required to narrow the design space to a feasible range. The number of samples and resolution of the composition-property relations (i.e., step size in the concentrations of individual components) are also user-defined parameters that may be influenced by the application. Here, the formulation was performed in commercial 96- and 48-well plates to demonstrate that the automated dispensing process is compatible with conventional platforms for HTE. The robot path associated with the automated formulation corresponded to a 96- or 48-point dispense command that was executed in an N-shaped path that corresponded to the dimensions (e.g., well spacing) of the given well plate. The dispense time (i.e., dwell time) per well varied for each solution and desired mass dispensed.

The movement speed of the dispensing nozzle between each well was 10 mm/s. A new dispensing nozzle was used for each experiment.

#### **4.2.2.1 Automated Robotically-directed Mixing**

Following the dispensing process and prior to the crosslinking (i.e., synthesis) process, the dispensed components in each well were mixed by various strategies, including diffusion, a digital plate shaker (LSE digital microplate shaker; Corning), and robotically-directed mixing. Hydrogel libraries that contained alginate-based precursor solutions, which were relatively viscous, were mixed by a robot-assisted mixing method using the same dispensing robot. The mixing pattern in each well was controlled using a circular band motion command, which generated a spiral path for mixing with a given brush area. The mixing time varied between 30 and 75 s. The gap between the stirrer and the bottom of the well plate was 0.1 mm. Stir tools with straight cylindrical geometry (22, 20, and 18 gauge nozzles) and helical geometry (steel wire (diameter ( $d$ )  $\sim$  0.4 mm); inner helix  $d = 1.4, 1.7$ , and 2.0 mm; pitch  $\sim 1.5$  mm) were fabricated and affixed to unfilled dispensing barrels to facilitate robotically-directed mixing (see Figure SB - 1). Hydrogels that contained relatively less viscous precursor solutions with viscosity similar to DIW were mixed by a digital shaker at 1000 rpm for 2 min. Photographs of the mixed samples were acquired before and after mixing.

#### **4.2.2.2 PEGDMA Hydrogels**

A PEGDMA stock solution was prepared by dissolving 4.5 g PEGDMA in 24.73 mL of DIW, followed by mixing with 0.77 g of 1 wt% DMPA solution in ethanol, which resulted in a stock precursor solution that contained 15 wt% of PEGDMA and 0.5% DMPA with respect to the PEGDMA molar concentration. The stock solution was then filtered through a 0.45  $\mu$ m nylon syringe filter to remove large particles. 96-Well plates were used as reaction vessels for high-

throughput formulation, synthesis, and characterization. The precursor solution was dispensed using a straight cylindrical nozzle (32 gauge). The 96 samples were formulated with the PEGDMA stock solution using a dispensing time (i.e., dwell time) per well that ranged across the plate from 0 to 47.5 s with a step size of 0.5 s. To complete the formulation of the hydrogel library, DIW was dispensed in opposite path direction (i.e., beginning in the last well and traveling to the first well) with dwell time ranging from 47.5 to 0 s with a step size of 0.5 s. After dispensing, the samples were then mixed with a digital shaker at 1000 rpm for 2 min prior to crosslinking. The PEGDMA hydrogels were then obtained by exposure of the samples (i.e., well plate) to UV light for 30 min (365 nm; UVGL-58; Analytik Jena US LLC).

#### **4.2.2.3 Alginate/PNIPAM Hydrogels**

A 2 wt% alginate precursor solution was obtained by dissolving alginic acid sodium salt in DIW. A 100 mM  $\text{CaCl}_2$  in DIW served as the stock crosslinking solution. The PNIPAM precursor solution was prepared by dissolving NIPAM and MBAA in DIW followed by mixing with 16  $\mu\text{L}$  of 1 wt% DMPA solution in ethanol, which resulted in a stock precursor solution that contained 6.7 wt% of NIPAM in which the molar ratio between NIPAM, MBAA, and DMPA was 1:0.005:0.0005, respectively. The NIPAM and  $\text{CaCl}_2$  solutions were separately filtered by a 0.45  $\mu\text{m}$  nylon syringe filter prior to dispensing. The alginate precursor solution was first dispensed using a straight cylindrical nozzle (27 gauge) for a dispense time of 10 s in each well. Then NIPAM solution was then dispensed using a straight cylindrical nozzle (32 gauge) for a dispense time that ranged from 0.5 to 48 s with an increment of 0.5 s per well. DIW was dispensed in an opposite path direction for a dispense time that ranged from 48 to 0.5 s to achieve a constant total mass in each well. The samples were then mixed via robotically-directed mixing for 56 s per well using the helical stirring tool. The samples (i.e., well plate) were then exposed to UV light (365 nm) for

30 min to polymerize the PNIPAM network of the composite hydrogel. Finally, 30.7 mg of  $\text{CaCl}_2$  solution was then dispensed on the surface of each sample using a circular band path trajectory to chemically crosslink the alginate network of the composite hydrogel. The alginate/PNIPAM composite hydrogels were then obtained by allowing the crosslinking reaction to proceed for 12 h in a humid environment.

#### **4.2.2.4 PEGDMA/PEDOT:PSS Hydrogels**

A stock PEGDMA precursor solution (25 wt%) was first dispensed using a straight cylindrical nozzle (27 gauge) for 3 s in each well. The vendor-provided PEDOT:PSS formulation (1.3 wt%) served as the PEDOT:PSS precursor solution and was then dispensed in each well with a dispense time of 0 to 23.5 s and a step size of 0.5 s. The stock DMPA precursor solution in ethanol (10 wt%) was then dispensed for 3 s in each well. The precursor solution was then mixed with the digital shaker at 500 rpm for 5 min. Finally, the precursor solution was photopolymerized by exposure to UV light (365 nm) for 45 min.

### **4.2.3 Automated HTC of Hydrogel Properties in Well Plate Formats**

Multiple properties of the synthesized hydrogel libraries, including mechanical, optical, and electrical properties, were characterized in well plate formats using various automated HTC techniques.

#### **4.2.3.1 HTC of Hydrogel Rheological Properties**

Rheological properties of the synthesized hydrogel libraries were characterized in high-throughput via robotically-directed sensing using piezoelectric milli-cantilever (PEMC) sensors (see Figure SB - 1) and a recently reported method<sup>136</sup>. The PEMC sensor fabrication method and measurement principle can be found in our previous reports.<sup>116, 142-145</sup> PEMC sensor phase angle at resonance, which correlates with the material shear storage modulus ( $G'$ ),<sup>116, 142, 143</sup> was continuously

monitored using an impedance analyzer (e5061b; Keysight) and custom Matlab program for data acquisition. The path planning parameters for HTC in 96-well formats, which included a 30 or 60 s dwell time per well, is provided in our recent report.<sup>136</sup> Rheological property characterization data and corresponding heat maps are presented as the steady state of the phase angle at resonance in each material (i.e., well). After all samples were characterized, the sensor signal was acquired in air for 30 min to re-establish the sensor baseline after testing.

#### **4.2.3.2 HTC of Hydrogel Electrical Properties**

The electrical properties of the synthesized hydrogel libraries were characterized via robotically-directed sensing using a robot (F5200N; Fisnar) and impedance analyzer (e5061b; Keysight). Electrochemical impedance spectroscopy (EIS) was performed at 3 kHz with zero DC bias using a two-electrode setup (see Figure SA - 1; electrode 1 = platinum (d = 1 mm; Sigma); electrode 2 = platinum (d = 0.25; Alfa Aesar)). The electrode separation distance was 4 mm. The path trajectory and path planning parameters were identical to those used for HTC of rheological properties. EIS characterization data are presented as the steady-state electrical impedance (Z) in each well. The electrical impedance obtained using the impedance analyzer was benchmarked against a potentiostat (Interface 1000; Gamry) by collecting EIS data across the 0.1 – 100 kHz frequency range using a two-electrode setup, identical electrodes and a stimulus of 10 mV AC and zero DC bias. The electrical impedance at 3.144 kHz was used for benchmarking.

The conductivity ( $\sigma$ ) of the samples was obtained using an electrometer (Keithley 6514; Tektronix) using a custom mold (width (W) = 4 mm; height (H) = 3 mm; and length (L) = 40 mm) and a parallel plate (copper) electrode configuration, which yielded the resistance (R). Multiple measurements were performed at three different sample lengths (20, 30, and 40 mm) to verify that

the contact resistance ( $R_{\text{contact}} < 1\%R$ ) was neglectable compared to the bulk resistance. The conductivity was calculated as  $\sigma = L/(RWH)$ .

#### **4.2.3.3 HTC of Hydrogel Optical Properties**

Optical properties, specifically absorbance (A) at 450 nm, of the synthesized hydrogel libraries were measured using a microplate reader (Synergy H1; BioTek Instruments). Measurements were first obtained at room temperature and subsequently at 37 °C by immersing the well plate in a water bath until the samples reached thermal equilibrium, after which the absorbance was measured.

#### **4.2.4 3D Printing of Conductive Hydrogels with Optimized Formulation**

The formulation of the PEGDMA/PEDOT:PSS composite hydrogel for optimized processability and performance identified by HTC of mechanical and electrical properties, respectively (i.e., the formulation that provided a balance of  $G'$  and conductivity ( $\sigma$ )), was utilized for 3D printing. The optimized precursor solution formulation contained 13.1 wt% PEGDMA, 0.4 wt% PEDOT:PSS, and 0.2 wt% DMPA. Silicone support structures were printed in the shape of a Virginia Tech logo (i.e., adjacent “V” and “T” characters) on a glass slide. The optimized precursor solution was then printed layer-by-layer in the support structure. After each layer was printed, the dispensed solution was cured by exposure to UV light (365 nm) for 10 min. The cured composite hydrogel construct was then removed from the support structure. The two electrodes of a light-emitting diode (3-3.2V forward voltage; MCIGICM) were then inserted in the constructs (i.e., one electrode was inserted into each character – the characters were otherwise not in contact). Copper wires under each of the characters (one per character) served as the interconnect to a direct current (DC) power supply (GPD-2303S; GW Instek). The copper interconnects contacted only the bottom surface of the hydrogel to avoid direct contact with the LED electrodes that penetrated the top surface of the

hydrogel. Photographs of the circuit composed of the LED and conducting composite hydrogel were obtained between 5 - 8 V. A second circuit composed of LEDs and cured composite hydrogel constructs was also fabricated. Multiple rectangular constructs (13 x 7 x 2 mm<sup>3</sup>) were prepared by molding. The constructs and five LEDs were then connected in series in an alternating configuration to form a “V” character. The bottom surfaces of the two terminal hydrogel constructs were contacted with copper foil to facilitate connection to the DC power supply. Photographs of the circuit composed of the five LEDs and hydrogel interconnects were obtained between 14 and 16 V.

#### **4.2.5 Data Analysis**

All experiments were repeated at least three times. The reported results correspond to the mean and standard deviation of all experiments. Details associated with conversion of PEMC sensor data to mechanical property information (i.e., G') can be found in our previous work and Supplementary Material.<sup>142, 144</sup>

### **4.3 Results and Discussion**

#### **4.3.1 High-Throughput Formulation of Hydrogel Libraries via Robotically-directed Multi-material Dispensing**

While several reports have been made on HTS and HTC,<sup>61, 62, 64, 65, 70-72, 74, 77, 135</sup> it remains a goal to establish integrated HTS and HTC processes that can autonomously operate in a closed loop with minimal human intervention (e.g., manual operations or human decision-making). In addition, there remains a need for accelerated materials discovery workflows for functional soft materials that support both established and emerging areas, including tissue engineering and soft robotics, respectively. HTE requires three steps: formulation (i.e., selection and combination of components), synthesis (i.e., reaction of combined components), and characterization (i.e.,

analysis of the composition, structure, and properties of resultant product mixtures). As shown in Figure 4.1, here, we provide an automated HTE method for accelerated engineering and optimization of soft functional composite hydrogels based on robotically-directed dispensing and sensing.

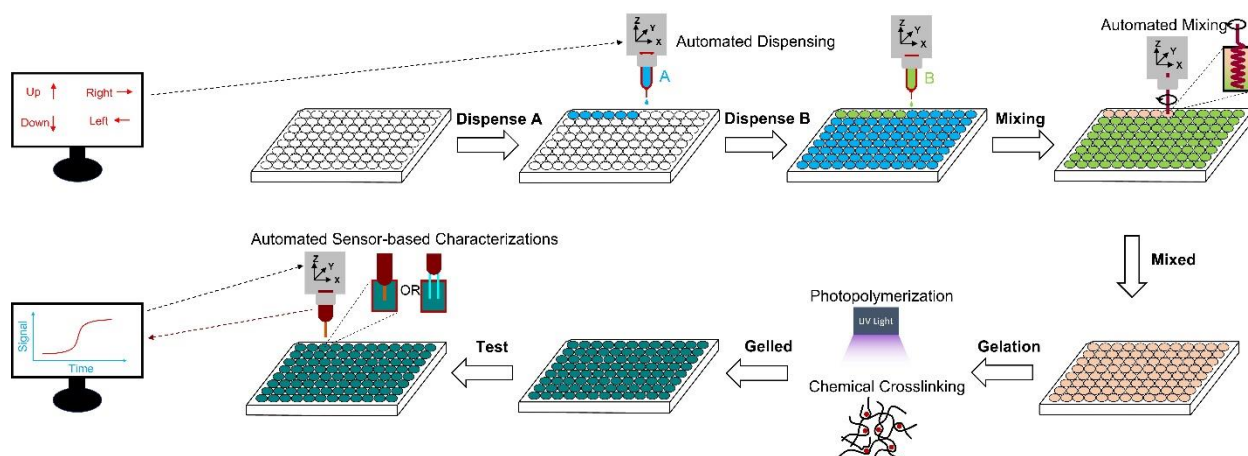


**Figure 4.1** Schematic of the high-throughput experimentation (HTE) method for accelerated engineering of functional composite hydrogels with optimized properties for processability and performance via integrated robotically-directed multi-material formulation, synthesis, and multi-property characterization in well plate formats.

We first demonstrated that robotically-directed dispensing enables high-throughput formulation of hydrogels by controlled integration of precursor solutions, crosslinking agents, and associated solvents (e.g., DIW). As shown in Figures 4.2 A and B, respectively, the mass of the dispensed liquid increased linearly with respect to the dispensing pressure and time. The method provided two dispensing regimes across the investigated pressure range, specifically, a droplet-based regime and a continuous-flow regime. A dispensing pressure of 6.5 psi was the transition point of the two regimes. Linear relationships between the dispensed mass and dispensing pressure and time were consistent across the two regimes. Given the volume of liquid in the dispensing

container (i.e., syringe) decreases throughout the formulation process, we next investigated the influence of the dispensed liquid volume on the dispensed mass. As shown in Figure 4.2 C, we found that the process enabled controlled dispensing as the volume of the dispensing container decreased. Figure 4.2 C shows that the change in mass dispensed associated with the volume decreases that occurred during the high-throughput formulation studies in this work, which ranged from 0.3 to 18.3 mL, was minimal and quantifiable.

As shown in Figure 4.2 D, a batch of samples with varying mixture composition, referred to as a library, can be formulated by successive dispensing of multiple stock solutions that exhibit different components or concentration in varying spatial distributions and volumes across a well plate. The stock solutions can represent precursor solutions, crosslinking agents, or solvents. Figure 4.2 D illustrates the process of formulating a hydrogel library by dispensing two solutions, such as a concentrated precursor solution and a diluent. For example, component A is dispensed from low to high mass across the well plate with respect to ascending well number, while component B is dispensed with from low to high mass in the opposite path direction (i.e., with respect to descending well number). The process was demonstrated using a dye-containing solution and DIW, which represent components A and B, respectively (see Figure 4.2 E). The ability to control the mass of the dispensed solutions across a 96-well plate in nonlinear spatial distributions was demonstrated in Figure 4.2 F by formulating a Virginia Tech logo (i.e., “V” and “T” characters).

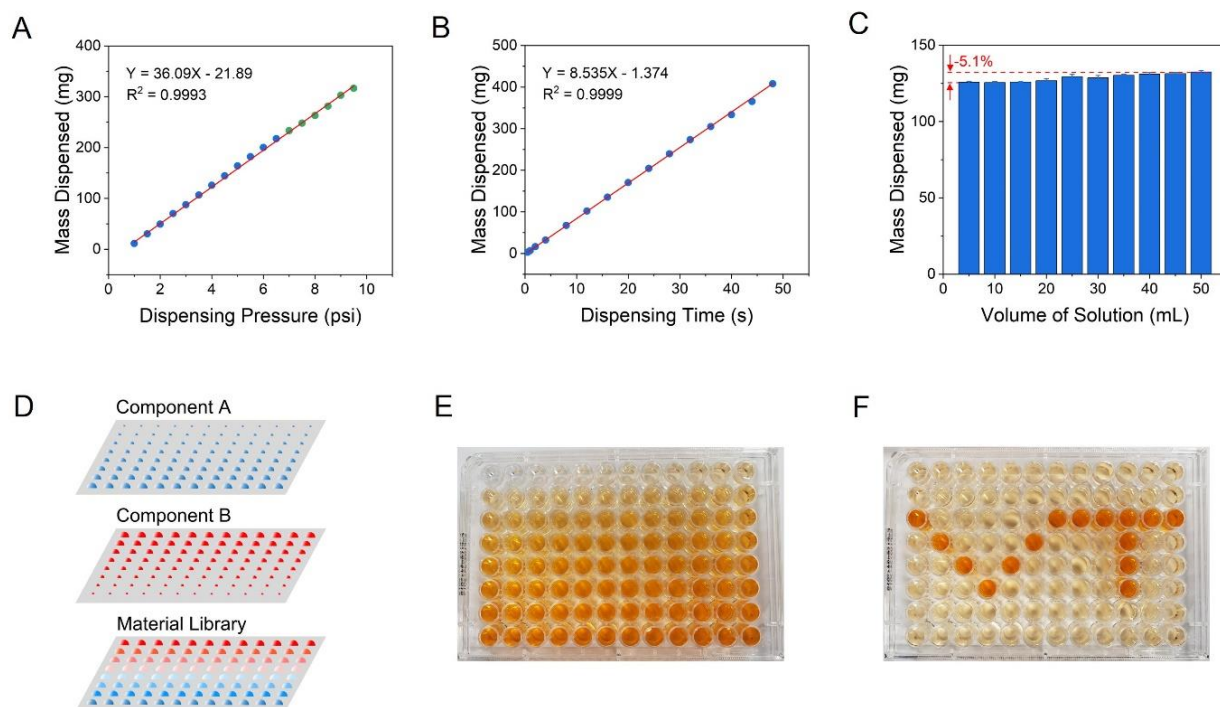


**Figure 4.2** Relationship between the mass of dispensed solution and dispensing pressure (A) and dispensing time at a constant dispensing pressure (4 psi) (B) (blue and green points represent droplet-based and continuous-flow dispensing regimes, respectively). (C) Effect of volume decrease in the dispensing container during formulation on the mass dispensed at constant dispensing pressure and time (4 psi and 15 s, respectively). Volume decreases in this study ranged from 0.3 to 18.3 mL. (D) Illustration of formulating a batch of hydrogel samples of varying composition via spatially-controlled multi-material dispensing. Photographs representing formulated hydrogel libraries in a 96-well plate format using an orange dye with a linear distribution in mixture composition (E) established by the method shown in panel (D) and nonlinear distribution in mixture composition (F).

#### **4.3.2 Automated Mixing of Formulated Hydrogel Libraries Containing Viscous Precursor Solutions**

In addition to the automated formulation of batches of samples with varying mixture composition, mixing of the formulated samples is a critical aspect of HTS, particularly when considering accelerated discovery and optimization of bulk materials. For example, HTS of material libraries that contain samples with spatially non-uniform distributions of reactants may affect the accuracy and fidelity of composition-structure-property relations that are obtained via subsequent HTC. Given that composite hydrogels are often synthesized by combining several precursor solutions, crosslinking agents, and solvent, which may exhibit different density and viscosity, validation of mixture mixing is an important step associated with HTS of composite hydrogels. While mixing may be achieved by diffusion in microarray- and microfluidic-based formats for HTS of hydrogels, it may be insufficient for HTS of bulk composite hydrogels. Thus, additional mixing techniques, such as shaking<sup>65</sup> and sonication<sup>77</sup>, have been implemented for HTS applications in well plate formats. At present, most reported studies on HTS of hydrogels have utilized precursor solutions of relatively low viscosity similar to DIW. However, the use of relatively high-density and -viscosity precursor solutions increases the demand for both establishing novel in situ mixing techniques and validating sample mixing prior to synthesis steps. Multi-material printing processes that require mixing of printed materials may be accomplished in-line,<sup>146</sup> but also be constrained by sample size, discrepancy of the printed material rheological properties, and kinetics of hydrogel gelation. While in-line mixing of dispensed or printed components prior to deposition has opportunities for creating blends and gradients in material composition throughout a construct, in-line mixing techniques based on static mixers have the drawback of material residence time, which limits low-volume applications. Here, we did not constrain processing in the well plate format with in-line mixing strategies during dispensing based on the synergy of well plates with various

mixing processes. Thus, we next examined a well plate-compatible robotically-directed high-throughput mixing technique and benchmarked the performance (i.e., mixing time and efficiency) vs. standard mixing techniques of diffusion and plate shaking.

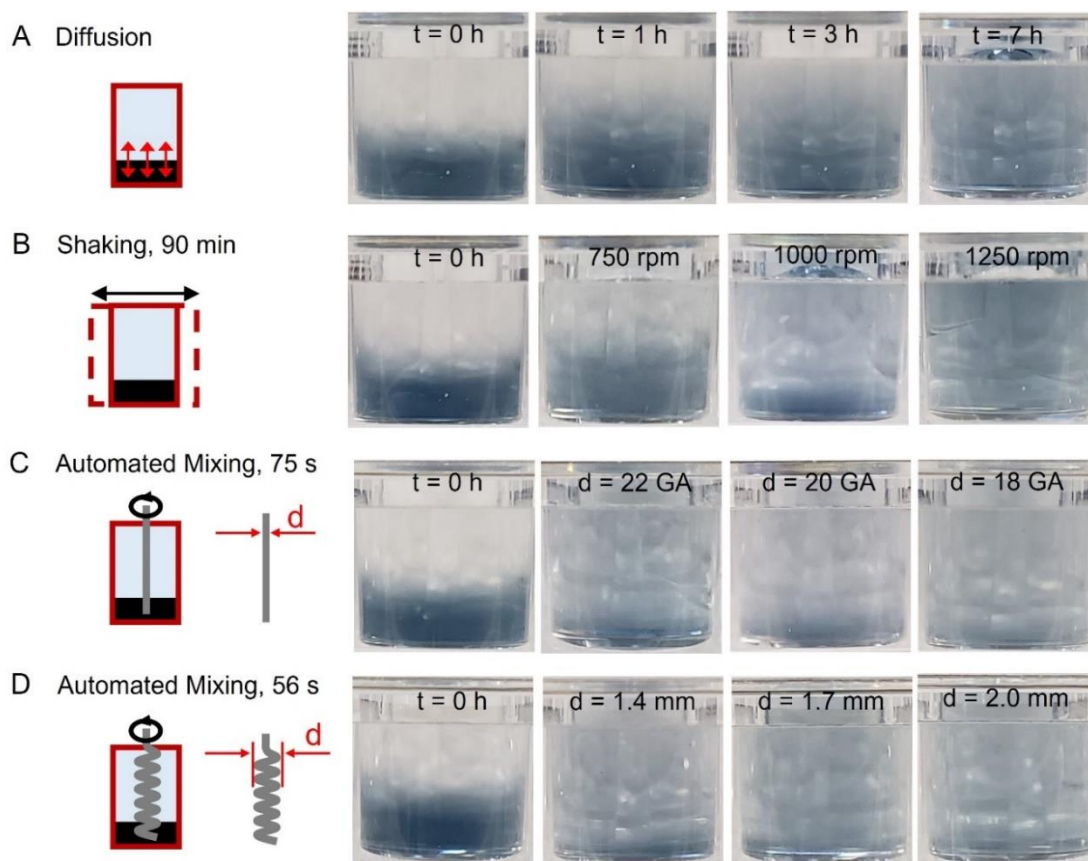


**Figure 4.3** Comparison of a robotically-directed mixing method with established mixing methods using a viscous alginate/PEDOT:PSS precursor solution and diluent solution. (A) Photographs of the mixing efficiency achieved by diffusion for 1, 3, and 7 h. (B) Photographs of the mixing efficiency achieved by a digital plate shaker for 90 min at 750, 1000, and 1250 rpm. (C) Photographs of the mixing efficiency achieved by robotically-directed mixing for 75 s with the straight cylindrical stirring tool (22, 20, and 18 gauge nozzles). (D) Photographs of the mixing efficiency achieved by robotically-directed mixing for 56 s with a helical stirring tool ( $d = 1.4$ ,  $1.7$ , and  $2.0$  mm).

A comparative study was performed by observing the mixing between a viscous and an inviscid precursor solution, represented by an alginate/PEDOT:PSS precursor solution and DIW, respectively. Alginate/PEDOT:PSS solution (100  $\mu$ L; 2 wt%) was first added to a well, followed by DIW (290  $\mu$ L). The viscosity of the 2 wt% alginate stock solution used in this research was 977.9 mPa s. As a comparison, 25 wt% PEGDMA aqueous solution, which exhibits a viscosity similar to many common precursor solutions, was 8.3 mPa s. The viscosities of several other solutions used in this work are also provided in Table SB - 1 of Supporting Information. As shown in Figure 4.3 A, diffusion was unable to rapidly establish a uniform mixture (i.e., mixture with uniform spatial distribution of components). In contrast, plate shaking for 90 min established a uniform mixture (see Figure 4.3 B). Similarly, we found that robotically-directed mixing for approximately one minute per well also established a uniform mixture. Figures 4.3 C and D compare the effect of the stirring tool geometry on the required mixing time. We found that the required time to achieve a uniform mixture correlated with the surface area of the stirring tool, which is consistent with a drag-based mixing mechanism. Given that the robotically-directed mixing method required  $\sim$  1 min per well, the total mixing time and quality were comparable to plate shaking; however, we note that multiplexed stirring tools may be developed that facilitate simultaneous mixing of multiple wells, which can potentially reduce the mixing time for the library to as low as 1 min, which would be a significant improvement relative to plate shaking. In practice, the mixing method utilized (e.g., diffusion, plate shaking, robotically-directed mixing) is likely application and material dependent (e.g., dependent on required throughput and properties of integrated components, respectively). However, it is critical to examine methods that can potentially decrease the cycle time associated with the mixing process as well as to validate that samples generated by high-throughput formulation processes exhibit uniform mixtures prior to

synthesis. Efficiency is also important for high-throughput experimentation as effective mixing of reagents prior to, and sometimes during, the reaction is a critical aspect of creating sample quality and reproducibility. Overall, there is a goal to optimize the efficiency (i.e., reduce cycle times) of formulation, mixing, reaction, and characterization processes to improve the cycle rate of high-throughput experimentation processes. The choice of mixing technique is dependent on various factors, including the differences in density, viscosity, and miscibility of dispensed solutions, the relative rates of phase separation (if any) and reaction, sample size, crosslinking method and parameters, and required sample quality. Here, our goal was to demonstrate that a combination of viscous and inviscid solutions or suspensions in a well plate format by multi-material dispensing can be actively mixed following dispensing, which could reduce the time-to-results relative to diffusive mixing or sonication.

#### **4.3.3 HTC of Hydrogel Mechanical Properties**

Having validated the high-throughput formulation process for HTS of hydrogels in well plate formats, we next demonstrated the integrated HTS and HTC of a highly utilized hydrogel system (PEGDMA hydrogel).<sup>147-149</sup> As shown in Figures 4.4 A and B, the frequency response of a PEMC sensor across the 30 – 60 kHz frequency range, which contained a resonant mode, facilitates characterization of the hydrogel mechanical properties via a material-structure interaction mechanism. For example, as shown in Figure 4.4 B, the sensor phase angle at resonance was dependent on the hydrogel's PEGDMA concentration. The raw data associated with HTC of the hydrogel library mechanical properties via robotically-directed sensing is shown in Figure 4.4 C. The sensor response associated with the measurement of a single sample contains three phases: a penetration phase, a measurement phase, and a removal phase. A zoomed-in response of the sensor

phase angle at resonance is shown in the lower right-hand corner of the panel, which highlights the measurement of three adjacent samples.

As shown in Figure 4.4 C, the steady-state sensor phase angle at resonance in each sample exhibited a nonlinear decrease with respect to the PEGDMA concentration across the 0 – 15 wt% concentration range. Comparison of the sensor baseline before and after the study as well as before and after individual sample measurements indicates both minimal drift in the response and absence of material adhesion during characterization of successive samples. The sensor baseline drift was examined as a negative control (see Figure SB - 2). The sensor response recovered after each measurement cycle (i.e., penetration and removal event) to  $99.97 \pm 0.19\%$  of the original baseline. The baseline drift ranged among multiple sensors was  $-0.08 \pm 0.17\%$  ( $n = 4$  repeated tests using two different sensors), which was significantly lower than the signal change associated with the HTC studies (for example, Figure 4.2 C). Figure 4.4 D shows the composition-property relation generated from the raw HTC data using a fluid-structure interaction model<sup>142, 150</sup> as a relationship between  $G'$  and PEGDMA concentration. The fluid-structure interaction model allows measurements of the sensor's resonant frequency and quality factor, which is correlated with phase angle at resonance, to be converted to viscoelastic properties of the surrounding materials. The correlation of the viscoelastic properties measured at the resonant frequency using the cantilever sensor with low-frequency values obtained with traditional rheology and dynamic mechanical analysis has been previously established.<sup>116, 142, 143</sup> The fluid-structure interaction model is further described in Supporting Information. The nonlinear trend of  $G'$  vs. PEGDMA concentration exhibits three regions with transition points near 4 and 14 wt%. Initially,  $G'$  increases gradually at a constant rate with increasing PEGDMA until the first transition point near 4 wt% after which  $G'$  increases at a relatively higher rate with nonlinear dependence on PEGDMA concentration until

$G'$  ultimately stabilizes near 14 wt% (the second transition point). The first transition point is associated with gelation of the mixture and the second is associated with a plateau in the hydrogel  $G'$ . Similar characteristics of the sol-gel transition have been reported in several hydrogels and composites<sup>151, 152</sup>. Thus, the reported HTE method offers new opportunities for studying the phase behavior and composition-property relations of hydrogels.

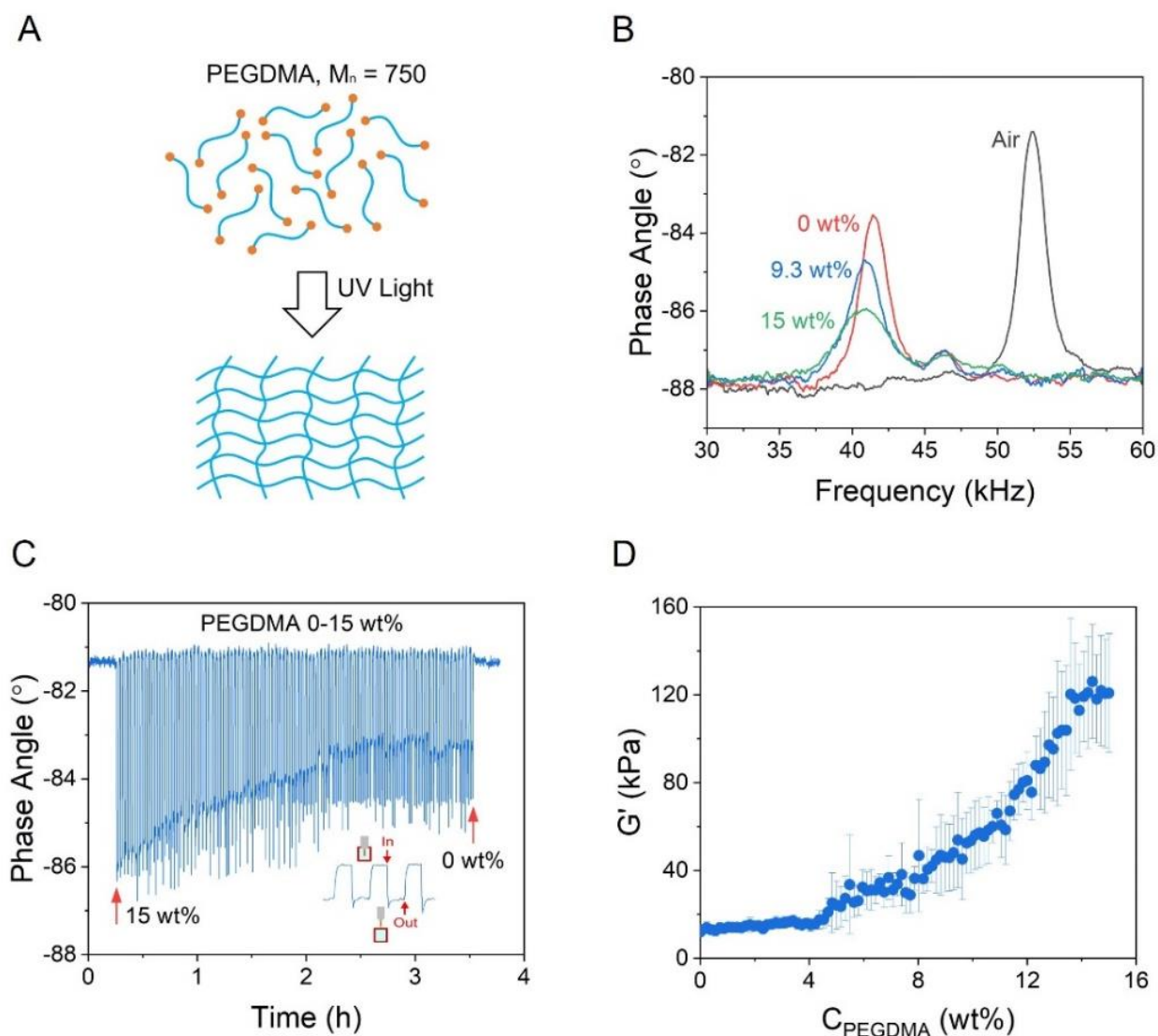


**Figure 4.4** (A) Illustration of the photopolymerization process for PEGDMA hydrogel gelation. (B) Dielectric impedance spectra (phase angle response) of PEMC sensors across the 30-60 kHz

frequency range in air, DIW, 9.3 wt% PEGDMA hydrogel, and 15 wt% PEGDMA hydrogel. (C) Real-time phase angle at resonance response (raw HTC data) associated with HTC of mechanical property HTC data for PEGDMA hydrogels in a 96-well plate format ranging from 0 to 15 wt%. (D) The generated composition-property relation data for PEGDMA hydrogels in terms of shear storage modulus ( $G'$ ) dependence on PEGDMA concentration associated with the raw HTC data shown in panel (C).

#### **4.3.4 HTE with High-throughput Multi-property Characterization of Stimuli-responsive Composite Hydrogels**

Having established the ability to study the phase behavior of hydrogels using HTC of mechanical properties, we next extended the HTE method to characterization of composition-multi-property relation data. The demand for accelerated discovery and engineering (e.g., optimization) of functional materials remains a driving force for the MGI. However, optimizing the formulation of functional materials requires multi-property information to assess both functionality (i.e., performance) and processability.<sup>153-155</sup> Often, there is a tradeoff between a material's processability and performance, and therefore, an optimized formulation should yield resultant properties that enable optimized processability and performance. Thus, we next examined the integration of complementary tools and methods for HTC of hydrogel optical and electrical properties with the established HTE method. Given the use of stimuli-responsive alginate/PNIPAM composite hydrogels for a range of applications, including soft robotics and 4D printing, and the ability to characterize the rheological properties of alginate/PNIPAM composite hydrogels using PEMC sensors,<sup>144</sup> we next leveraged the HTE method for multi-property HTC of alginate/PNIPAM composite hydrogel libraries. Figure 4.5 A illustrates the process of alginate/PNIPAM composite hydrogel formation. A mixture of alginate and NIPAM monomer solutions is first exposed to UV light to photopolymerize a PNIPAM network. Subsequently, the mixture is exposed to a  $\text{Ca}^{2+}$ -

containing solution to form an interpenetrated alginate network. The concentration of alginate in all samples was 0.5 wt%, while the concentration of PNIPAM varied from 0.03 to 4.57 wt%. Figure 4.5 B shows the sensor phase angle at resonance response across the library, which exhibited a similar trend to that observed for the PEGDMA hydrogel library. The first transition point was observed near 2.5 wt% PNIPAM. The corresponding dependence of  $G'$  on the PNIPAM concentration is shown in Figure 4.5 C and exhibited a similar trend. Likewise, the first transition point was observed near 2.5 wt% PNIPAM.

It is established that robust networks can enhance the strength of interpenetrated hydrogel networks.<sup>156</sup> While there are several methods to characterize the structure and properties of hydrogels, such as scanning electron microscopy (SEM), small angle neutron scattering (SANS), and rheometry,<sup>96, 157-160</sup> most are incompatible with well plates. In contrast, plate readers are mature well plate-compatible HTC platforms that are widely used for materials science, chemistry, biochemistry, and biology applications.<sup>161-164</sup> As shown in Figure 4.5 D, the composite hydrogel exhibited relatively low absorbance at room temperature (RT) regardless of the PNIPAM concentration below the lower critical solution temperature (LCST) but exhibited a sigmoidal trend above the LCST, a characteristic of percolation.<sup>165</sup> When the PNIPAM concentration was below a threshold value (2.56 wt%), relatively weak absorbance was observed both below and above the LCST. The HTC data associated with the composite hydrogel optical properties (Figure 4.5 D) was correlated with the HTC data associated with the composite hydrogel mechanical properties (Figure 4.5 C) and can be utilized to interpret the composition-mechanical property relation data. For example, the data suggest that when the PNIPAM concentration was below 2.56 wt%, the covalent PNIPAM network was unable to reinforce the ionic alginate network, and the phase separation was weak when the temperature was above the LCST. In contrast, when the PNIPAM

concentration was greater than 2.56 wt%, the PNIPAM network was capable of forming an effective interpenetrated network with the alginate network that both enhanced the mechanical properties and caused phase separation when the temperature was above LCST. Similar transitions have also been reported in other double-network hydrogels.<sup>166, 167</sup> The results shown in Figure 4.5 suggest that the reported HTE method can potentially enable accelerated engineering (e.g., optimization) of stimuli-responsive hydrogels for a range of applications. These results also demonstrate the value of leveraging complementary composition-property relations for data interpretation.

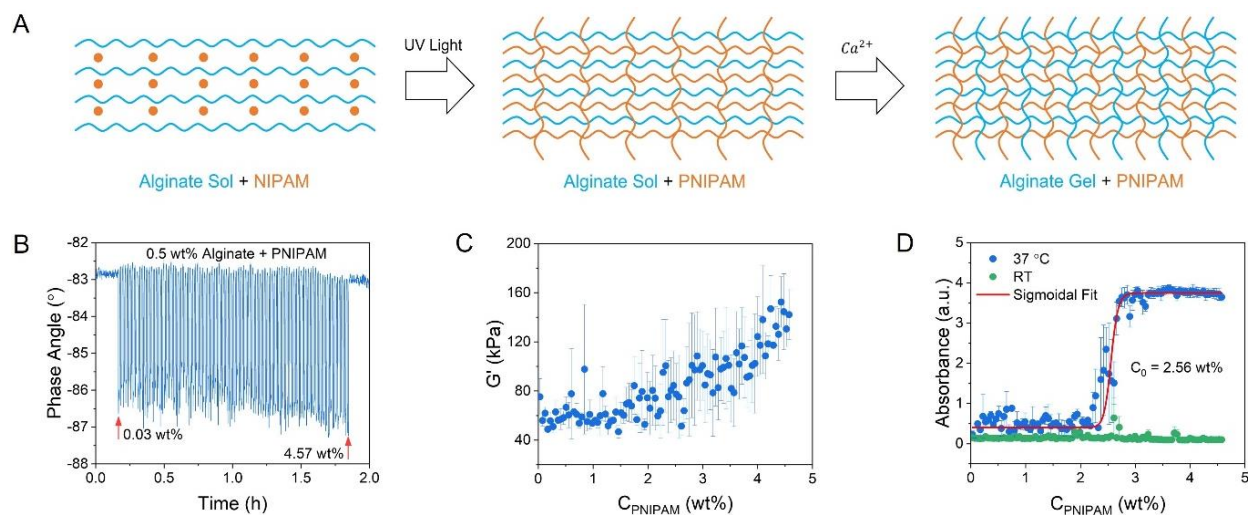


**Figure 4.5** (A) Illustration of alginate/PNIPAM composite hydrogel formation via sequential photopolymerization of PNIPAM and chemical crosslinking of alginate. (B) Real-time phase angle at resonance response (raw HTC data) associated with mechanical property HTC data for alginate/PNIPAM composite hydrogels in a 96-well plate format. (C) Composition-mechanical property relation data in terms of PNIPAM concentration and  $G'$  of alginate/PNIPAM composite hydrogels corresponding to the data shown in panel (B). (D) Composition-optical property relation

data in terms of PNIPAM concentration and absorbance (450 nm) of alginate/PNIPAM composite hydrogels below (room temperature) and above the LCST of PNIPAM (37 °C).

#### **4.3.5 Accelerated Engineering of Optimized Functional Composite Hydrogels via HTE with High-throughput Multi-property Characterization**

Having demonstrated the utility of the HTE method for understanding the phase behavior and formulation optimization of a stimuli-responsive composite hydrogel via multi-property HTC, we next leveraged the method for understanding the phase behavior and formulation optimization of a conductive functional composite hydrogel. The conductive polymer PEDOT has been widely used for electronics applications.<sup>168-171</sup> However, the introduction of PEDOT, especially PEDOT:PSS particles, into another material may affect the mechanical properties of the resultant composite material and, thus, the performance and processability.<sup>172</sup> It remains a challenge to establish optimized formulations that may balance performance and processability metrics, such as conductivity and elasticity. Thus, we next applied the HTE method with integrated multi-property HTC to optimize a conductive functional composite hydrogel system, specifically, a PEGDMA/PEDOT:PSS composite hydrogel (see Figure 4.6 A). The PEGDMA concentration was constant at 13.1 wt% for all samples, while PEDOT:PSS concentration increased from 0 to 0.6 wt%. A photograph of the library and representative samples after manual removal from the well are shown in Figure 4.6 B. Visual inspection of representative samples removed from the well plate after synthesis revealed that increasing PEDOT:PSS concentration increased the sample brittleness, resulting in sample fracture by manual handling. Importantly, prior to removal and manual handling for photography purposes all hydrogels exhibited similar geometry. Raw data associated with the rheological and electrical properties HTC process are shown in Figures 4.6 C and D, respectively. As shown in Figure 4.6 E, there was a tradeoff between the mechanical and electrical properties (i.e., elasticity and conductivity, respectively). The composite hydrogel G'

exhibited three regions (strong, transition, weak) and was significantly reduced at PEDOT:PSS concentrations above 0.4 wt%. For PEDOT:PSS concentrations below 0.4 wt%, increasing PEDOT:PSS concentration caused an increase in conductivity and a decrease in electrical impedance but had relatively little effect on the hydrogel elasticity. For PEDOT:PSS concentrations above 0.45 wt%, increasing PEDOT:PSS concentration caused continued conductivity increase but at a relatively lower rate of change while the elasticity (i.e.,  $G'$ ) remained relatively constant. However, between the two regions (i.e., 0.4 - 0.45 wt%)  $G'$  underwent a sharp change, specifically, a significant decrease with respect to increasing PEDOT:PSS concentration. Benchmarking of the electrical impedance response obtained using a network analyzer vs. potentiostat is shown in Figure 6.F with the associated material conductivity (Bode plots associated with potentiostatic EIS data are shown in Figure SB - 3A). The measured resistance of the samples also exhibited a trend similar to that of the electrical impedance data (see Figure SB - 3B). The multi-property HTC data show a clear tradeoff between mechanical and electrical properties and can be used to identify an optimized formulation for device manufacturing. For example, while the conductive functional composite hydrogel may be relatively more conductive above PEDOT:PSS concentrations of 0.45 wt%, it exhibits poor mechanical properties. Thus, we selected a PEDOT:PSS concentration of 0.4 wt%, the concentration that precedes the sharp decrease in mechanical properties, as an optimized formulation for subsequent device fabrication via additive manufacturing. Thus, the selection of the optimized formulation here was based on the generated composition-property relations in combination with imposed thresholds on material property-based critical quality attributes. Figures 4.6 G and H show photographs of 3D-printed PEGDMA/PEDOT:PSS composite hydrogel constructs in the shape of a Virginia Tech (“VT”) logo containing integrated LEDs. The minimum voltages required to generate light visible with the

naked eye for the two circuits were approximately 4.5 and 14 V, respectively. Composite hydrogels of PEGDA/PEDOT have many potential applications, including bioelectronics.<sup>172-177</sup> These applications require the composite material to exhibit suitable mechanical and electrical properties, which can be identified via HTE-based accelerated materials optimization.

Considering material optimization applications of the HTE method, which was the focus of this work, existing composition-process-property data from previous studies can provide design constraints for the formulation (e.g., concentration limits for specific mixture components). However, for material discovery applications in which the composition-property relations may not yet have been investigated for many materials, the HTE method can leverage knowledge generated by initial manual experimentation to avoid blind investigation of the material design space (i.e., composition-process parameter space). For example, traditional experimentation may identify limits on reagent composition (e.g., formulations that do not solidify). Active learning is also increasingly used to guide HTE processes for accelerated material discovery and optimization applications to overcome the challenges associated with blind exploration of the formulation-process parameter design spaces in cases of limited prior knowledge and complex material systems. In this work, the experimental design was constrained and guided by the prior knowledge of the material system's phase behavior, processability (e.g., minimal dispensing volume), sensor characteristics (e.g., detection limit), sample number associated with well-plate formats, and property of interest to be optimized, which here was most typically the mechanical property. Beyond high-throughput mechanical property characterization, other high-throughput characterization measurements, such as the multi-property characterization of optical and electrical properties, assisted in understanding the mechanical property-composition relation. For example, the change of absorbance of the alginate/PNIPAM material helped to understand the percolation

of the interpenetrated polymer network as well as the trend in mechanical property-composition relation observed by the cantilever sensor data (i.e., phase angle response).

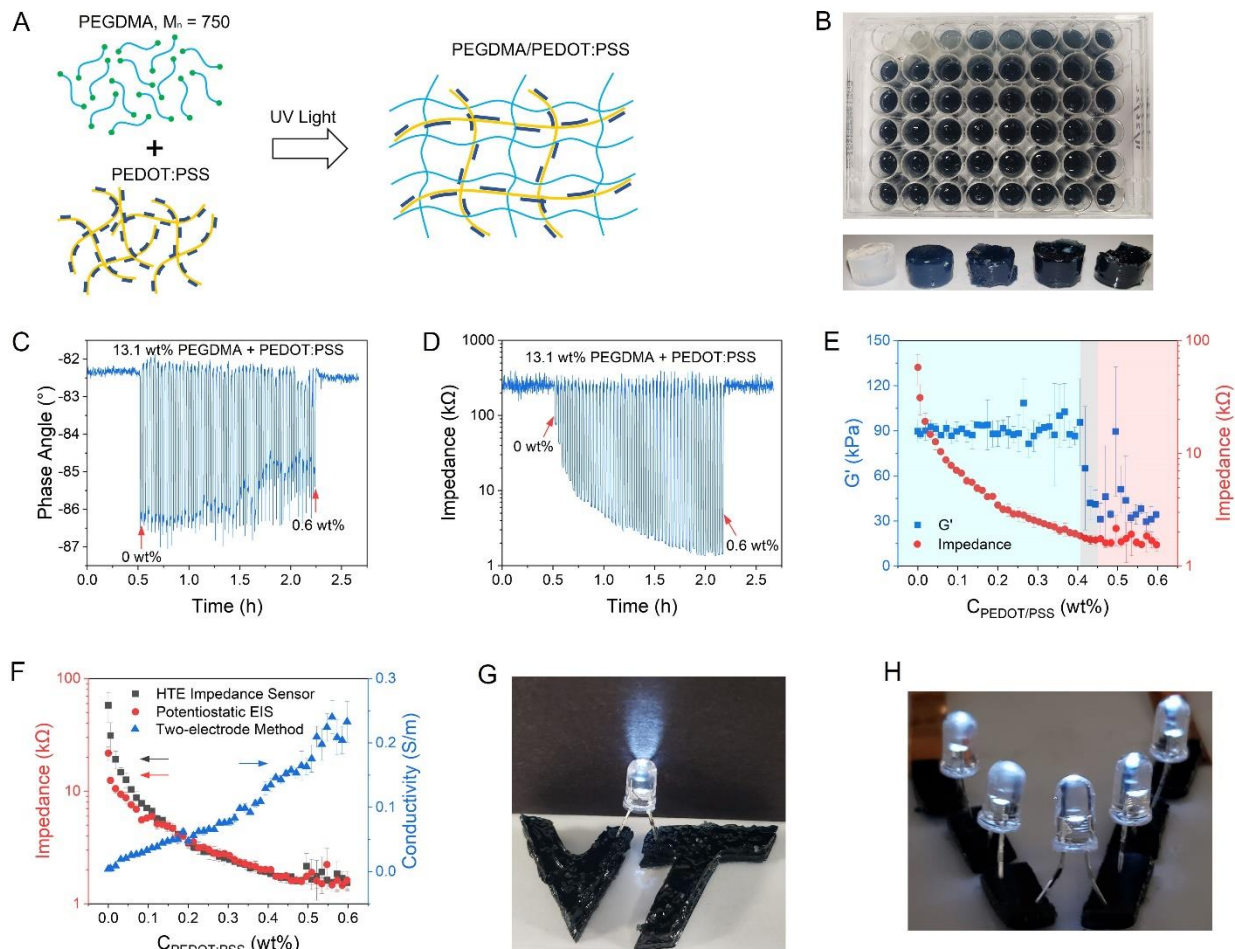


**Figure 4.6** (A) Illustration of formation of PEGDMA/PEDOT:PSS conductive composite hydrogels via photopolymerization. (B) Photographs of a formulated PEGDMA/PEDOT:PSS hydrogel library in a 48 well plate (upper panel) and representative samples containing 0, 0.09, 0.19, 0.36, and 0.56 wt% of PEDOT:PSS after manual removal from the well plate (lower panel). The samples highlight the brittleness of hydrogels composed of relatively high PEDOT:PSS concentration (all samples exhibited consistent geometry before manual removal). Real-time phase angle at resonance response associated with HTC of mechanical ( $G'$  - panel (C)) and

electrical (impedance ( $Z$ ) – panel (D)) properties of a 48 sample PEGDMA/PEDOT:PSS composite hydrogel library. (E) Composition-multi-property relation data for the mechanical (i.e.,  $G'$ ) and electrical (i.e.,  $Z$ ) properties of PEGDMA/PEDOT:PSS composite hydrogels with respect to PEDOT:PSS concentration. (F) Composition-electrical property relation data in terms of electrical impedance obtained by multiple HTC methods and conductivity with respect to PEDOT:PSS concentration. (G) Photograph of a 3D-printed PEGDMA/PEDOT:PSS composite hydrogel construct and integrated LED fabricated using an optimized formulation (13.1 wt% of PEGDMA and 0.4 wt% of PEDOT:PSS). (H) Photograph of multiple PEGDMA/PEDOT:PSS composite hydrogel constructs in series with five LEDs fabricated using the optimized formulation.

#### **4.4 Conclusions**

This work was driven by the demand for accelerated engineering (e.g., optimization) of soft functional materials, particularly hydrogels, which are used extensively. There remains a need for HTE formats that synergize with multi-property HTC methods for accelerated engineering of functional composite hydrogels with optimized processability and performance, particularly in practical cross-disciplinary formats, such as well plates, that are used extensively in HTE applications. We demonstrated a HTE method based on integrated robotically-directed dispensing and sensing that synergizes with well plate formats. The method was leveraged to identify formulations of several functional composite hydrogels that exhibit optimized processability and performance. Limitations of the method are rooted in the performance constraints of the underlying dispensing and sensing tools. Thus, the performance of the HTE method can be improved in future work with advances in the underlying processes and tools (e.g., parameter selection, path planning, and sensing). In application to other material systems, one may consider material availability (sample size limitations) and solubility, which may constrain the

concentration range investigated. Material selection may also be constrained by material processability (e.g., extrudability and miscibility). The concentration range may also be constrained by the relationship of the material's properties with respect to the dynamic range of the utilized characterization tools (e.g., detection limit). Future opportunities include application to continuous monitoring of hydrogel dynamic processes (e.g., gelation and stimuli-response), understanding temperature dependence of material properties, integration with digital twin, and incorporation with data-driven decision making. The ultimate goal is to utilize generated HTC data to establish autonomous closed-loop HTE formats that can operate with minimal resource expenditures and human intervention.

## **5 Scalable Accelerated Materials Discovery by Collaborative Learning**

While it is now possible to rapidly discover and engineer materials using closed-loop workflows, such as those based on high-throughput experimentation and active learning, scaling of self-driving accelerated materials discovery (AMD) workflows remains challenging. Coordinating the activity of multiple clients (i.e., agents) and workflows toward a common objective may provide novel opportunities for scalability and performance optimization. Here, we describe a methodology for high-performance, scalable AMD based on high-throughput experimentation and collaborative learning via consensus Bayesian optimization (BO), built upon seeking optimal collaboration among multiple clients (e.g., experimental AMD workflows) during the iterative design process. The impact and utility of the methodology were illustrated by the rapid discovery of mechanically optimized composite glycogels. The methodology's performance was compared with that achieved by a non-collaborating AMD workflow based on the trend of mechanical property evolution over eight experimental iterations. Notable performance was obtained following five iterations. Ultimately, we show that collaborative learning by consensus BO can enable scaling and performance optimization of self-driving AMD workflows.

### **5.1 Introduction**

The Materials Genome Initiative (MGI) aims at accelerating the pace and reducing the cost of materials discovery and engineering. Materials innovation infrastructures, including computational, data, and experimental tools and processes, are needed to achieve this goal.<sup>3, 178</sup> Integration of materials innovation infrastructures is central to advancing performance. For example, the performance of experimental processes and tools for materials science and engineering research can be transformed by the integration of simulation and data science.

Automation and optimization have played a significant role in advancing high-throughput experimentation (HTE), such as helping establish combinatorial and closed-loop workflows.<sup>9, 42, 46, 49, 179-185</sup> In particular, machine learning (ML) and artificial intelligence (AI), which are founded on optimization, now facilitate autonomous AMD.<sup>186-194</sup> For example, ML and AI contribute to high-performance (e.g., efficient and autonomous) AMD workflows by data analysis and sequential design.<sup>195</sup>

Several examples of closed-loop and autonomous AMD workflows have been provided, such as based on self-driving simulation or experimentation.<sup>196</sup> While AMD can be achieved by brute force using HTE systems driven by automation,<sup>196</sup> the high-dimensionality of the composition-process-property relations associated with practical functional materials, which are often based on multi-component mixtures and complex processing operations, has driven the creation of AMD workflows based on adaptive sampling.<sup>195, 197 198-200</sup>

Both Bayesian optimization (BO) and active learning use adaptive sampling to achieve a specific learning goal.<sup>201</sup> BO is a sequential process that aims to reduce the number of required experiments by optimizing an expensive black-box (unknown) function,<sup>202</sup> such as the true relationship between the material composition and properties, which is typically performed by costly experimentation. The BO process begins by using a few initial data points to learn a surrogate model, often a Gaussian Process (GP), that approximates the black-box function.<sup>203</sup> A property of a GP is its intrinsic ability to quantify uncertainty.<sup>204</sup> Subsequently, BO relies on a utility function, constructed based on the surrogate, to quantify the potential benefits of conducting experiments with a new material formulation. Through optimizing the utility function, which balances exploration and exploitation, one can select a new formulation for subsequent characterization that will best reveal the desired, yet unknown, composition-process-property

relation. Exploration refers to allowing the for new feature discovery about the environment, while exploitation refers to capitalizing on knowledge already gained. This labeled data is then integrated with the existing dataset, and the cycle is then repeated over multiple iterations until available resources (e.g., budget) are utilized or an exit condition is met. Burger et al. implemented BO on a mobile robot to autonomously search for photocatalysts with improved hydrogen production performance.<sup>205</sup> Shields et al. used BO to identify the top-yielding conditions in the Mitsunobu reaction and deoxyfluorination reaction processes.<sup>206</sup>

Active learning, sometimes referred to as ‘query learning,’ is the study of ML systems that improve by asking questions.<sup>207</sup> Active learning is particularly useful when unlabeled data are numerous, labeling consumes significant resources (e.g., experimentally), or it is anticipated that many data must be labeled to train a model.<sup>207</sup> There are many different ways by which a learner can ask queries. Kusne et al. used Bayesian active learning to discover phase-change memory material by identifying the composition-phase structure relation over 19 experimental iterations.<sup>208</sup> Min et al. used active learning to discover inorganic materials based on efficient global optimization (EGO) to identify materials satisfying band gap and refractive index over 50 experimental iterations.<sup>209</sup> Ohtelie et al. used active learning to design layered materials using Bayesian optimization was used to find optimal structures with respect to band gap and electronic fitness function values.<sup>210</sup>

While adaptive sampling methods can improve the efficiency of AMD workflows, it is prudent to consider scalability. For example, it is desirable to leverage multiple AMD systems, such as HTE systems, toward a common AMD objective, such as by hierarchical modeling.<sup>211</sup> Unfortunately, despite its utility in AMD, BO operates in isolation and lacks a collaborative

element. Thus, there remains a critical need to integrate principles of collaboration within BO and active learning methods to achieve scalable AMD across multiple distributed systems.

With today's advances in computation and communication power at edge devices (e.g., computers, laptops)<sup>212</sup>, it has become more plausible for multiple clients (e.g., human experimentalists or automated AMD systems) to share information, distribute trial and error efforts, and fast-track the experimental process so that all participants gain benefit, which is a key element for AMD performance and scalability. Here, we demonstrate a methodology for scalable, high-performance AMD driven by collaborative learning. Specifically, we demonstrate the utility and impact of a consensus BO framework where multiple collaborating clients perform experiments locally and agree (i.e., reach a consensus) on their individualized 'next-to-test' formulations, thereby achieving scalability through a shared AMD objective. Through this research, we aim to showcase the potential of merging collaborative ML with HTE to expedite and scale material discovery initiatives.

## **5.2 Experimental Section**

### **5.2.1 Materials**

Acetic acid (AcOH) was from Sigma-Aldrich. Multi-reducing-end alginate (M-alginate) and carboxymethyl chitosan sodium salt (CMCS) were synthesized as previously reported.<sup>213 214</sup> Lead zirconate titanate (PZT-5A,  $72.4 \times 72.4 \times 0.127$  mm<sup>3</sup>) with nickel (Ni) electrodes was from Piezo Systems (Woburn, MA). Glass cylinders and ethanol (200 proof) were from Fisher Scientific. Polyurethane (Fast-Drying) and Loctite EA 1C-LV epoxy adhesive were from Minwax and Henkel, respectively. De-ionized water (DIW; 18.2 M $\Omega$ ) was from a commercially available system (Direct-Q® 3 UV Water Purification Systems; Millipore).

### 5.2.2 Collaborative and Distributed BO with Consensus

We briefly define mathematical notation that will be used. Assume there are  $K$  clients (e.g., experimenters, such as standalone HTE systems), and each client has a budget of  $T$  experiments, which is a reasonable assumption given experiments consume resources and total resources available are often limited. Let  $t \in \{0, 1, \dots, T-1\}$  be the iteration index. Consider  $\mathcal{D}_k^{(0)} = \{X_k^{(0)}, y_k^{(0)}\}$  the initial dataset with  $N_k^{(0)}$  samples, where  $X_k^{(0)} = (x_{k,1}, \dots, x_{k,N_k^{(0)}})$  is a matrix that contains the initial input vector data  $x_{k,\cdot}$  (i.e., material composition, specifically the concentration of each component in the mixture) and  $y_k^{(0)} = (y_{k,1}, \dots, y_{k,N_k^{(0)}})^\top$  is a vector that contains the corresponding outputs (i.e., numeric measures of the material properties for each composition, such as the shear storage modulus ( $G'$ )). Here, the input data  $x_{k,\cdot}$  is of dimension  $D$ , where each dimension denotes a component in the material (i.e.,  $D$  = the number of different species in the mixture). The goal of each client is to find a formulation (i.e., mixture composition) that optimizes a target property of the material, such as  $G'$ , which is a measure of the stress required to deform the material under dynamic loading. This problem is somewhat analogous to a team of cooks attempting to identify the ingredients of a recipe that generates optimized taste. Mathematically, this translates to the following optimization problem:

$$x_k^* = \operatorname{argmax}_x f_k(x) \quad (1)$$

where  $f_k$  is the true black-box function each client aims to optimize. To observe  $f_k(x)$  using a new formulation  $x$ , we need to conduct an experiment and observe a potentially noisy outcome  $y_k(x)$ .

In other words,  $y_k = f_k + \epsilon_k$ , where  $\epsilon_k$  is an additive noise.

In an environment where clients operate independently, each client selects successive formulations to test by maximizing its own utility without engaging in any form of communication

with other clients. More specifically from a mathematical perspective, at each iteration  $t$  (i.e., experiment number), a GP surrogate model  $\hat{f}_k$  is fit to the data  $\mathcal{D}_k^{(t)}$  to estimate  $\hat{f}_k(x)$  for any input  $x$ . Importantly, a GP provides a predictive distribution  $\mathbb{P}_{\hat{f}_k(x)|\mathcal{D}_k^{(t)}}$  over  $\hat{f}_k(x)$ . Equipped with the predictive distribution, the  $k$ -th client then chooses the next formulation to test by maximizing their own expected utility  $x_k^{(t)new} = \mathbb{E}_{\mathbb{P}_{\hat{f}_k(x)|\mathcal{D}_k^{(t)}}} \left[ U(\hat{f}_k(x); \mathcal{D}_k^{(t)}) \right]$ , where  $U(\hat{f}_k(x); \mathcal{D}_k^{(t)})$  is a utility function that quantifies the benefit gained if one were to conduct an experiment using a new formulation  $x$ , where  $x_k^{(t)new}$  is the recommended next-to-test formulation as it maximizes  $U$ . Many utility functions have been developed. Among the most used is the expected improvement (EI) utility,<sup>215</sup> which is defined as:

$$\mathbb{E}_{\mathbb{P}_{\hat{f}_k(x)|\mathcal{D}_k^{(t)}}} \left[ (\hat{f}_k(x) - y_k^{*(t)})^+ \right] = \sigma_k^{(t)}(x; \mathcal{D}_k^{(t)}) \phi(z_k^{(t)}(x)) + (\mu_k^{(t)}(x; \mathcal{D}_k^{(t)}) - y_k^{*(t)}) \Phi(z_k^{(t)}(x)) \quad (2)$$

where  $a^+ = \max(a, 0)$ ,  $y_k^{*(t)}$  is the current best observed output for client  $k$ ,  $\phi$  (or  $\Phi$ ) is a probability density function (or cumulative distribution function) of a standard normal random variable,  $z_k^{(t)}(x) = \frac{\mu_k^{(t)}(x; \mathcal{D}_k^{(t)}) - y_k^{*(t)}}{\sigma_k^{(t)}(x; \mathcal{D}_k^{(t)})}$ ,  $\mu_k^{(t)}(x; \mathcal{D}_k^{(t)})$  is the predictive mean of the GP at input  $x$  and  $\sigma_k^{(t)}(x; \mathcal{D}_k^{(t)})$  is the corresponding variance. EI can be interpreted as finding a new experimental formulation  $x$  that has the potential to improve upon the current best observed output. We note that surrogate modeling and utility optimization can be readily done using the GPytorch library.<sup>216</sup>

To foster collaboration and efficiently distribute the workload across multiple clients, we propose a ‘Collaborative BO’ approach, where clients reach a consensus on their next-to-test formulations. Through consensus, clients may borrow important information from each other to conduct more efficient and effective tests (e.g., experiments). Our consensus approach is accomplished through the application of a consensus matrix  $W^{(t)}$ , which regulates the extent to

which one client's decision influences the choices of others. From a mathematical perspective, the new objective function is as follows:

$$x_k^{(t)} = [x_c^{(t)}]_k = \underset{\hat{f}_k(x)|\mathcal{D}_k^{(t)}}{\operatorname{argmax}}_x \mathbb{E}_{\mathbb{P}} [U(\hat{f}_k(x); \mathcal{D}_k^{(t)})] \quad (3)$$

and

$$x_k^{(t) \text{new}} = [(W^{(t)} \otimes I)x_c^{(t)}]_k \quad (4)$$

where  $[\cdot]_k$  represents the  $k$ -th block of a vector,  $x_c^{(t)}$  is a vector that concatenates all  $x_k^{(t)}$  for  $k = 1, \dots, K$ ,  $W^{(t)}$  is a dynamic consensus matrix,  $\otimes$  is a Kronecker product operation, and  $I$  is an identity matrix. A key property of  $W^{(t)}$  is that it is a symmetric and doubly stochastic matrix (i.e.,  $\sum_k w_{kj}^{(t)} = \sum_j w_{kj}^{(t)} = 1$  for all  $j, k \in \{1, \dots, K\}$ ) with non-negative elements.

The consensus framework has several interesting features. First, it is clear from the consensus BO objective function (i.e., Equations (3) and (4)) that  $W^{(t)}$  plays a critical role in deciding the next-to-test formulation for each client  $k$  (i.e.,  $x_k^{(t) \text{new}}$ ). In other words,  $W^{(t)}$  controls the extent to which each client affects the decision of others. As a result,  $W^{(t)}$  adds a layer of flexibility to the optimization problem and allows a group of clients to determine the degree of collaboration dynamically with time (i.e., iteration number). Second, when  $W^{(t)}$  is an identity matrix  $I$ , the consensus step will keep the original vector  $x_c^{(t)}$  unchanged (i.e.,  $(W^{(t)} \otimes I)x_c^{(t)} = (I \otimes I)x_c^{(t)} = x_c^{(t)}$ ). In other words, this is equivalent to no information sharing, and therefore, the consensus BO model becomes traditional BO. Third, since  $W^{(t)}$  is doubly stochastic, this ensures that the formulations of all clients will stay in the solution space that has been explored. Finally, our consensus BO objective function generates an individualized solution for each client that allows the group of clients to distribute effort (e.g., experimental or computational), allowing parallel exploration-exploitation and more efficient experimental design. For instance, assume

$D = 2$ ,  $K = 2$ , and  $W^{(t)} = \begin{bmatrix} 0.6 & 0.4 \\ 0.4 & 0.6 \end{bmatrix}$ . Then if the local utility maximizers of the clients are  $x_c^{(t)} = [1, 2, 3, 4]^T$ , we obtain  $(W^{(t)} \otimes I)x_c^{(t)} = [1.8, 2.8, 2.2, 3.2]$  where  $x_1^{(t)new} = (1.8, 2.8)^T$  for client 1 and  $x_2^{(t)new} = (2.2, 3.2)^T$  for client 2.

The consensus BO objective functions were solved by the following steps:

1. At iteration  $t \in \{0, 1, \dots, T - 1\}$ , each client  $k$  solves  $\underset{\hat{f}_k(x) | \mathcal{D}_k^{(t)}}{\operatorname{argmax}} E_P [U(\hat{f}_k(x); \mathcal{D}_k^{(t)})]$  and obtains  $x_k^{(t)}$ .
2. Each client  $k$  sends its  $x_k^{(t)}$  to a central orchestrator.
3. The orchestrator concatenates all  $x_k^{(t)}$ 's, computes  $(W^{(t)} \otimes I)x_c^{(t)}$ , and then sends  $x_k^{(t)new}$  to the corresponding client  $k$ .
4. Each client  $k$  then conducts a test (e.g., experiment) using the new formulation  $x_k^{(t)new}$  and observes  $y_k(x_k^{(t)new})$ .
5. Each client  $k$  then augments the dataset  $\mathcal{D}_k^{(t)}$  by  $(x_k^{(t)new}, y_k(x_k^{(t)new}))$  to obtain a new dataset  $\mathcal{D}_k^{(t+1)}$ .
6. Each client  $k$  then updates its GP surrogate using  $\mathcal{D}_k^{(t+1)}$  and starts the new iteration  $t + 1$ .

The consensus BO framework naturally distributes effort (e.g., experimental) across different clients, allowing parallel exploration and exploitation and more efficient experimental design. Additional methodological details of the consensus BO methodology are provided in our previous work,<sup>217</sup> and our code is provided on GitHub<sup>218</sup>.

A key question that remains is how to design  $W^{(t)}$ . To enable effective collaboration,  $W^{(t)}$  is initialized as a uniform matrix whose all entries are equal. Mathematically, this is equivalent to:

$$W^{(0)} = \begin{bmatrix} \frac{1}{K} & \cdots & \frac{1}{K} \\ \vdots & \ddots & \vdots \\ \frac{1}{K} & \cdots & \frac{1}{K} \end{bmatrix} \quad (5)$$

The dynamic consensus matrix is then gradually shrunk to an identity matrix during the collaboration process. For example, if we adjust weights linearly:

$$W^{(t+1)} = W^{(t)} + \begin{bmatrix} \frac{K-1}{TK} & \cdots & -\frac{1}{TK} \\ \vdots & \ddots & \vdots \\ -\frac{1}{TK} & \cdots & \frac{K-1}{TK} \end{bmatrix} \quad (6)$$

The intuition behind this design is as follows. In the early stages, client  $k$  may not have enough observations to obtain a high-quality surrogate model and therefore needs to borrow information from other clients. In the late stages, as client  $k$  accumulates sufficient data and can construct a high-quality local surrogate model, it will focus more on its local problem to find client-specific optimal formulation.

### 5.2.3 High-throughput Synthesis of Composite Hydrogels

Hydrogels were fabricated using a previously reported HTE system based on robotic dispensing.<sup>196</sup> The precursor solutions were dispensed from a syringe barrel (55 or 10 mL) using a straight cylindrical nozzle (32 or 27 gauge). The two precursor solutions were dispensed in opposite path directions to achieve a complete material library. 48 samples were formulated with the 0.75 wt% M-alginate and 0.75 wt% CMCS precursor solutions with dispensing time per well ranging from 0 to 23.5 s and 23.5 to 0 s, respectively, using a step size of 0.5 s. M-alginate and CMCS precursor solutions were dispensed in opposite paths. The total concentration of M-alginate and CMCS in each sample (i.e., well) ranged from 0 to 0.66 wt% and 0.66 to 0 wt%, respectively, and the total solids concentration of each sample (i.e., well) was 0.66 wt% (composition of the hydrogel library see Table S1). DIW was subsequently dispensed across the formulated library to make the total mass of each sample identical (390 mg) after dispensing various masses of AcOH, which provided

a basis for the design objective. The precursor solutions in each well were then robotically mixed for 30 s using a helical stirring tool prior to dispensing acetic acid. Random and known volumes of acetic acid were then dispensed across the wells using three different stock concentrations of AcOH to cover a large portion of the material design space (i.e., to generate a wide composition range of the three components - M-alginate, CMCS, and AcOH). The dispensing time of AcOH (17.4 M) ranged from 0.8 to 10 s across the plate with a step size of 0.4 s. The dispensing time of 8.3 wt% and 0.69 wt% AcOH solutions ranged from 0.5 to 6 s with a step size of 0.5 s. The composite glycomaterial (M-alginate-CMCS) hydrogels (i.e., glycogels) were then obtained by allowing the crosslinking reaction to proceed for 12 h in a closed humid environment at room temperature. High-throughput characterization (HTC) of the 48 samples generated the training data for collaborative learning algorithm (i.e., to generate the composition recommendation for all clients for the 0-th iteration).

Material composition recommendations for all clients generated by the collaborative and non-collaborative learning algorithms (i.e., consensus BO and traditional BO) were formulated by sequential addition of M-alginate, CMCS, and DIW in 96-well plate. The sol mixture was then mixed using a digital plate shaker (LSE digital microplate shaker; Corning) at 700 rpm for 30 minutes (min). AcOH was then added to make the total mass of each sample identical to that used for the initial training library, and the samples were mixed for 5 min at 700 rpm. Finally, the 96-well plate was then placed in a closed humid environment for 12 hours to form the final hydrogels.

#### **5.2.4 High-Throughput Characterization of Composite Hydrogel Mechanical Properties**

The viscoelastic properties of the hydrogel samples, specifically  $G'$ , were measured in the 96-well plate format using a previously reported HTC system.<sup>136</sup> The dynamic-mode cantilever sensor fabrication method and measurement principle can be found in our previous reports.<sup>116, 136, 142, 144</sup>

Measurements were obtained using a 60 s dwell time per sample, which was sufficient to generate a steady-state sensor response. The sensor stabilization time between samples was 60 s. Following analysis of the last sample, the sensor signal was also continuously monitored to verify a stable baseline. The mechanical property of each sample, specifically  $G'$ , was calculated based on the steady-state value of the phase angle at resonance obtained during the dwell period. For each formulation, three sample replicates were prepared and simultaneously characterized. The reported results correspond to the mean and standard deviation of all measurements.

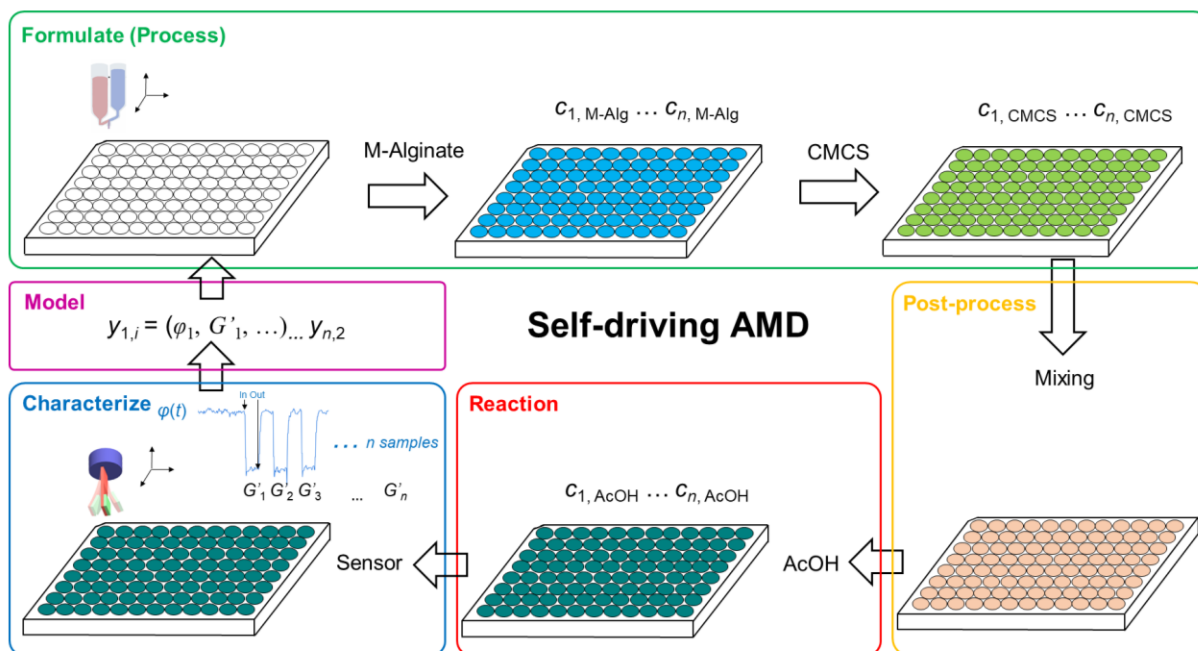


**Figure 5.1** Illustration of scalable self-driving workflow for AMD driven by high-throughput experimentation based on multi-material dispensing and automated sensing in well plate formats. A collaborative learning model facilitates selection of formulations to be tested among multiple collaborating clients (a detailed description of the collaborative learning model is provided in Figure 5.4).

## 5.3 Results and Discussion

### 5.3.1 Initializing the Collaborative Learning Process via Consensus BO for Scalable AMD using High-throughput Experimentation Data

As shown in Figure 5.1, the goal of this work is to generate a scalable self-driving workflow for AMD driven by high-throughput experimentation and collaborative learning. Alginate and chitosan are important renewable glycomaterials, which have been widely used as scaffolds for tissue engineering and drug release applications<sup>219-222</sup>. Alginate exhibits biocompatibility and tunable mechanical properties by controlling polymer content and crosslinking chemistry.<sup>223</sup> Chitosan is a glycomaterial that is compatible with many tissues in many situations, which offers attractive mechanical properties and reactive groups, such as for applications that require robust mechanical properties and chemical alteration.<sup>224</sup> M-alginate belongs to the family of multi-reducing end polysaccharides (MREPs) and forms hydrogels with CMCS. The reaction schemes to synthesize M-alginate and CMCS are shown in Figure 5.2 A. While M-alginate and CMCS form hydrogels (see Figure 5.2 B), it remains a challenge to engineer composite glycogels that combine the advantages of individual polysaccharides due to the challenges of blending glycomaterials. In addition, the currently unknown gelation mechanism of M-alginate-CMCS glycogels also motivates the use of AMD workflows to engineer and optimize the composite glycomaterial.

While considerable strides have been made in AMD of hard and brittle materials, such as using HTE, relatively less progress has been made in soft materials applications, such as hydrogels, due to challenges associated with adapting conventional methods for formulation, synthesis, and characterization of soft materials to high-throughput formats<sup>4</sup> as well as the high dimensionality associated with the design spaces of soft composite material. For example, solutions of both M-alginate and CMCS can form composite hydrogels in the presence of AcOH (see photographs in

Figure 5.2 B),<sup>225, 226</sup> but the mechanical properties of the hydrogel are highly dependent on the composition of the four component system (M-alginate, CMCS, AcOH, and water). Thus, composite M-alginate-CMCS hydrogels exhibit a high-dimensional design space, which presents a challenge for AMD, such as formulation discovery for optimal mechanical performance.

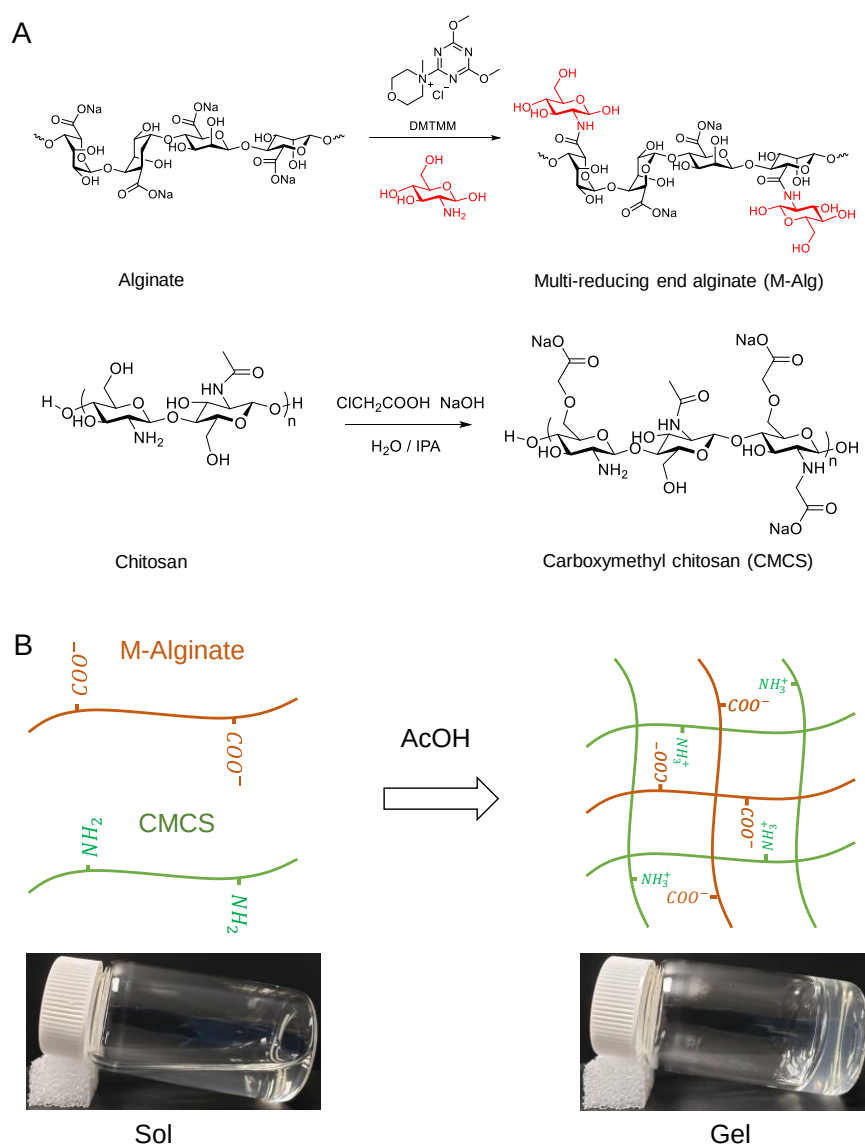


**Figure 5.2** (A) Schematics of synthesizing multi-reducing end alginate (M-alginate) and carboxymethyl chitosan (CMCS). (B) Illustration and photographs of M-alginate-CMCS

composite hydrogel gelation in the presence of acetic acid (AcOH) (photographs: left = sol; right = gel; M-Alg 0.37 wt%, CMCS 0.37 wt%, AcOH 0.50 wt%, Water 98.76 wt%).

The HTE process for synthesis and characterization the composite M-alginate-CMCS hydrogels is shown in Figure 5.1. Precursor solutions of M-alginate and CMCS were first dispensed and mixed. AcOH was then added, which catalyzed gelation,<sup>225, 226</sup> and  $G'$  of the composite hydrogels was then characterized by HTC in a 96-well plate format. Representative impedance spectra associated with the cantilever rheometer corresponding to the mixture in the sol and gel states are shown in Figure 5.3 A. As shown in Figure SC - 1, the spectra associated with three different formulations in the sol state were similar although the mixtures exhibited significantly different compositions (sol 1: 0.249 wt% M-alginate and 0.411 wt% CMCS; sol 2: 0.287 wt% M-alginate and 0.373 wt%; sol 3: 0.424 wt% M-alginate and 0.236 wt%, CMCS), indicating that the viscosity of mixtures were highly similar. In contrast, the spectra associated with the hydrogels formed by each of the formulations upon AcOH addition (sol 1: 5.187 wt% AcOH; sol 2: 7.738 wt% AcOH; sol 3: 6.237 wt% AcOH) were significantly different, which demonstrates the impact of the composite hydrogel formulation on  $G'$ . The hydrogel  $G'$  is correlated with the cantilever rheometer phase angle at resonance and can be quantified using the measured resonant frequency and phase angle at resonance using the fluid-structure interaction model of Mather *et al.*<sup>150</sup> The AcOH content is a critical formulation parameter as it catalyzes the reaction of amine groups of CMCS with aldehyde groups of M-alginate.

We first examined the dependence of the sol-gel transition on the AcOH concentration using a sample library in which the polymer content of all samples was constant. As shown in Figure 5.3 B, the relationship between the phase angle at resonance and the AcOH concentration exhibited a sigmoidal trend, providing the upper and lower bounds of AcOH mass (48 and 0.014 mg,

respectively) associated with the composite hydrogel formulation design space. Given that the composition-property relationship of the composite M-alginate-CMCS hydrogel exhibits a high-dimensional design space, we next initiated the scalable AMD process using HTE data generated from a 48-sample library of M-alginate-CMCS hydrogels. The polymer content of the samples was systematically varied and the AcOH content exhibited a random, yet known, distribution (see Figure 5.3 C), enabling the samples to span the associated phase diagram. In all samples, the total polymer mass was constant (i.e.,  $\text{wt\%}_{\text{M-alginate}} + \text{wt\%}_{\text{CMCS}} = 0.66 \text{ wt\%}$ ). The first and last samples in the library exhibited the lowest and highest relative amount of M-alginate, respectively. The first and last samples in the library exhibited the highest and lowest relative amounts of CMCS, respectively. The total mass of each sample, including M-alginate, CMCS, AcOH, and water, was 390 mg.

As shown in Figure SC - 2, the first 12 samples (i.e., hydrogels), which were rich in CMCS, exhibited phase separation, which further defined the boundaries of the high-dimensional design space. These compositions were eliminated from the subsequent studies as they exhibited inhomogeneity. Figure 5.3 D shows the raw HTC data associated with the other 36 samples, which formed homogeneous hydrogels. The corresponding steady-state phase angle at resonance of the 36 samples obtained by HTC is shown as a heat map in Figure 5.3 E. The random distribution of the heat map and absence of a sigmoidal trend as observed in Figure 5.3 B was an expected result and result of the experimental design (i.e., the samples were intentionally gelled by addition of a random distribution of AcOH - see Figure 5.3 C). The corresponding composition-property relation data for the acid-catalyzed composite M-alginate-CMCS hydrogel is shown in Figure 5.3 F as a ternary diagram in terms of  $G'$  dependence on the relative mass percentage of M-alginate, CMCS, and AcOH. The DIW content is not shown given the challenge associated with visualizing

the property dependence on the four-component mixture, and can be obtained for each sample based on the total mass of each sample, which was identical and served as a basis, and the known composition of all dispensed solutions. The DIW content of all samples is provided as Supplementary Material (see Table SC - 1).

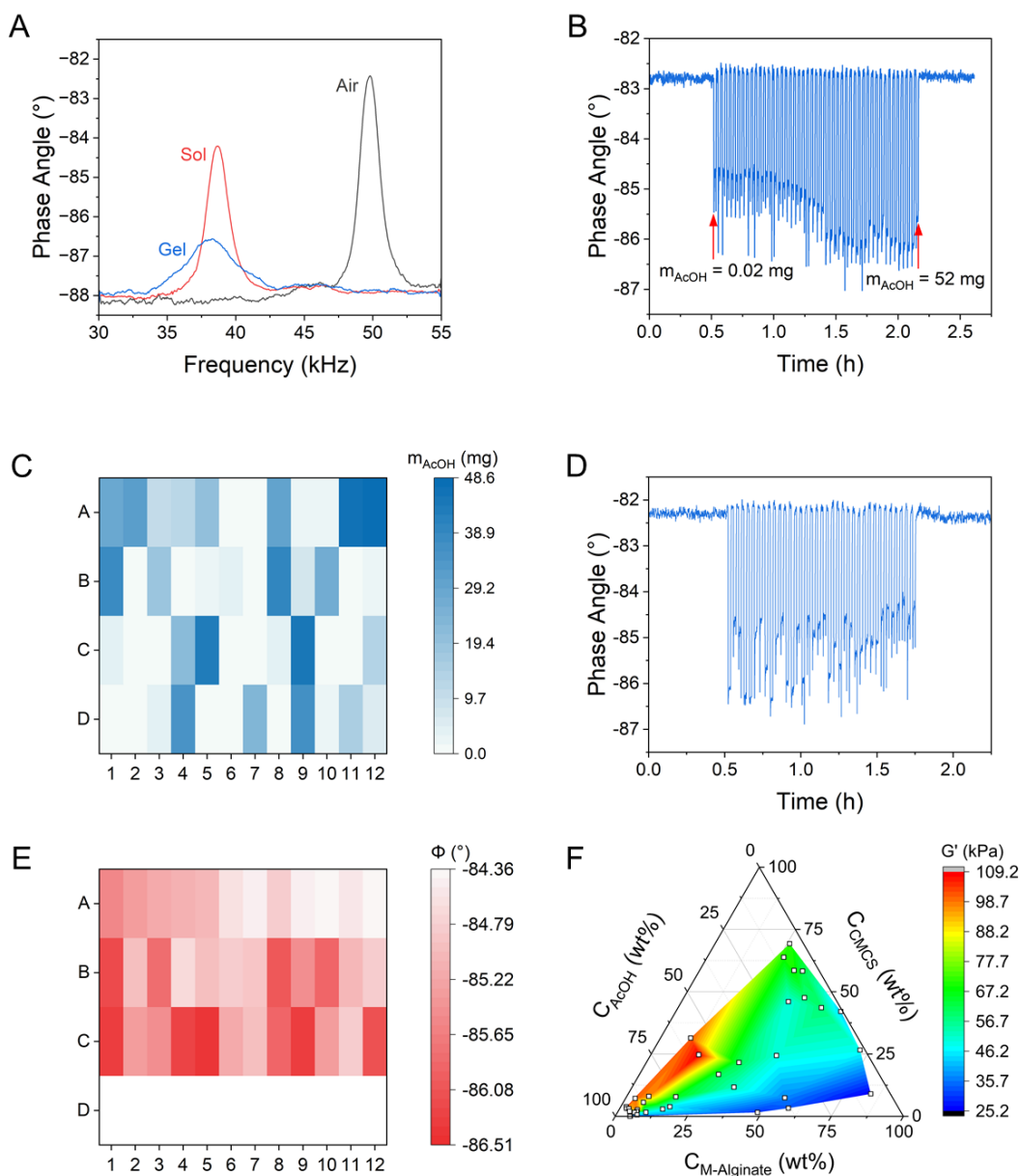


**Figure 5.3** (A) Impedance spectra of the cantilever rheometer upon submersion in a M-alginate-CMCS precursor solution (without AcOH) and resultant hydrogel formed by reaction with AcOH

for 12 hrs. (B) Results of a HTC study that established the upper and lower limits of the amount of AcOH used for crosslinking of the M-alginate-CMCS composite hydrogels. The concentration of the dispensed M-alginate and CMCS solutions were both 0.33 wt% and the mass of dispensed AcOH ranged from 0.02 to 52 mg. (C) Heat map showing the amount of AcOH dispensed in each sample in a 48 sample M-alginate-CMCS composite hydrogel library. (D) Real-time phase angle at resonance response (raw HTC data) associated with the M-alginate-CMCS composite hydrogel library in a 48-well plate format. (E) Heat map of the steady-state phase angle at resonance response across the library (12 samples were removed due to observed phase separation; thus, row D in the heat map does not contain sample information). (F) The generated composition-property relation for the M-alginate-CMCS composite hydrogel associated with the HTE study in terms of a ternary composition-property relation diagram (the water content in each sample is provided as Supplementary Material).

This study, which generated the training data for initialization of the collaborative learning algorithm, intentionally generated labeled data that spanned the high-dimensional design space. Relatively more labeled data was generated near the lower left corner of the diagram by design, given that the mass fraction of AcOH, which exhibited a maximum of 12.13 wt%, was higher than that of the polymer components, which exhibited a maximum of 0.65 wt%. The initial training data obtained by brute-force HTE suggest that an imbalance in the amount of the two biomacromolecular components in the mixture resulted in relatively weak hydrogels (i.e., hydrogels composed of excess M-alginate or CMCS appeared to form hydrogels of relatively reduced  $G'$ ). Furthermore, the results shown in Figure 5.3 also suggest that the mechanical properties of M-alginate-CMCS hydrogels (i.e.,  $G'$  and  $E'$ ) are highly dependent on the AcOH content. We remind the reader that the storage modulus ( $E'$ ) can be obtained from  $G'$  and the

Poisson ratio, which is typically assumed as 0.5 in hydrogels. Increasing the AcOH mass fraction increased the composite hydrogel  $G'$  regardless of the M-alginate:CMCS ratio.

### 5.3.2 Scalable AMD via Collaborative Learning using Consensus BO

Although HTE provides a methodology for exploring the high-dimensional design space and potential for AMD and material optimization, brute-force HTE may be prohibitive from a resource expenditure perspective. Also, there is no assurance that formulations identified by brute-force HTE that exhibit promising performance metrics are optimal. Rather, there is a significant opportunity to utilize HTE in combination with active learning for efficient and optimal AMD. Thus, we next used the labeled data generated by the initial HTE-driven AMD study to efficiently explore the material design space by collaborative learning through consensus BO. Figure 4A and the left panel of Figure 5.4 B summarize the AMD workflow based on collaborative learning via consensus BO. First, each client, indexed by  $k$ , initially optimizes its individual utility function to determine a candidate experimental formulation, which is stored in a vector  $x_k^{(t)}$ . These formulation vectors are then shared with a central orchestrator and concatenated into a vector  $x_C^{(t)} = \left(x_1^{(t)\top}, \dots, x_k^{(t)\top}\right)^\top$ . Subsequently, the consensus matrix  $W^{(t)}$  aggregates information from all clients. This operation results in a new vector  $x^{(t)new} = \left(x_1^{(t)new\top}, \dots, x_K^{(t)new\top}\right)^\top$  that contains personalized next-to-test formulations for each client. These next-to-test formulations (i.e., mixture compositions) are then shared by the orchestrator with the clients to test (e.g., via HTE). This process is then repeated until some exit condition is met (e.g., budget is exhausted) or convergence of a target property (e.g.,  $G'$ ) is achieved. In this context, convergence is determined by observing the trend of a target property over successive iterations. This distributed active learning approach driven by consensus BO transforms traditional BO to a new collaborative

paradigm, which offers potential to accelerate the optimal design process by effectively distributing experimental efforts across a network of AMD systems (see Figure 5.4).

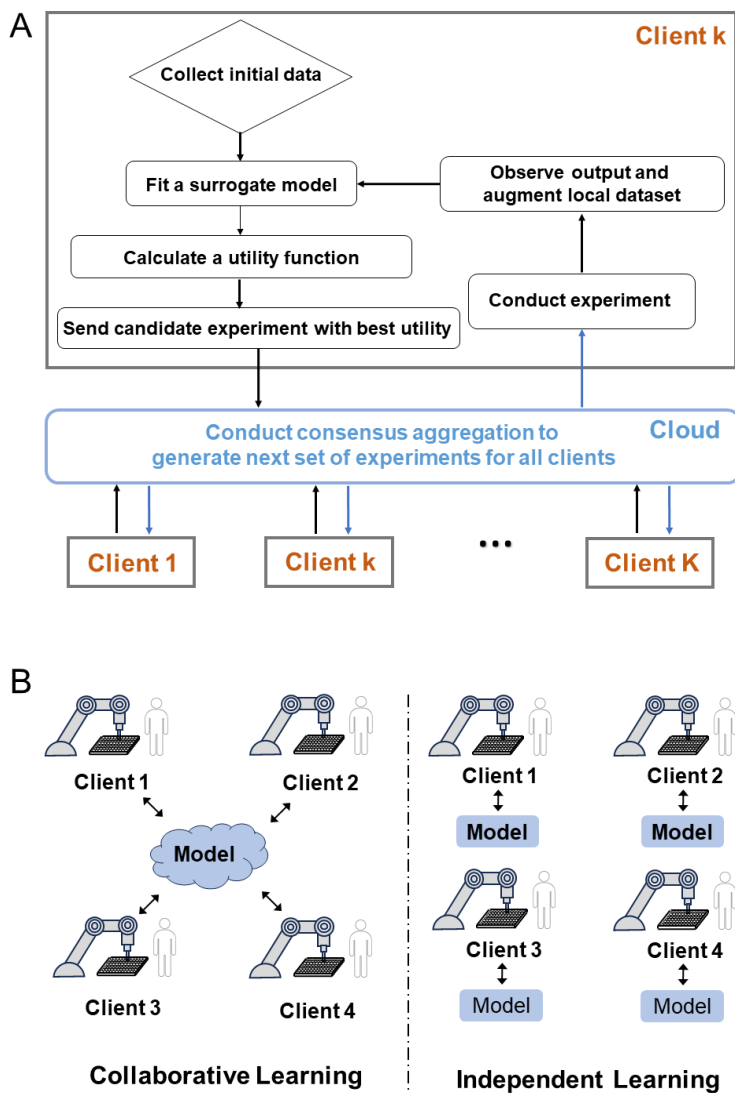


**Figure 5.4** (A) Flowchart for scalable accelerated materials discovery (AMD) via collaborative learning based on consensus BO using a network of clients (e.g., humans or automated AMD systems). (B) Schematics illustrating the difference of traditional non-collaborative BO and collaborative and distributed BO with consensus.

As shown in the leftt panel of Figure 5.4 B, the methodology was validated by consideration of four clients that share an AMD objective, specifically the objective to rapidly optimize  $G'$  of the composite M-alginate-CMCS hydrogel. A control group of four non-collaborating clients was also included (see right panel of Figure 5.4 B). Thus, each iteration of the collaborative learning algorithm generated eight formulations – four corresponding to the four collaborating clients and four corresponding to the four non-collaborating clients (i.e., each client examined one formulation per iteration). For each iteration, each client tested their formulation in triplicate, resulting in a total of 24 samples that were tested per iteration. HTC data associated with a single iteration (i.e., data from 24 samples) is shown in Figure SC - 3 as a representative case. Due to budget constraints, we limited the number of iterations to eight.

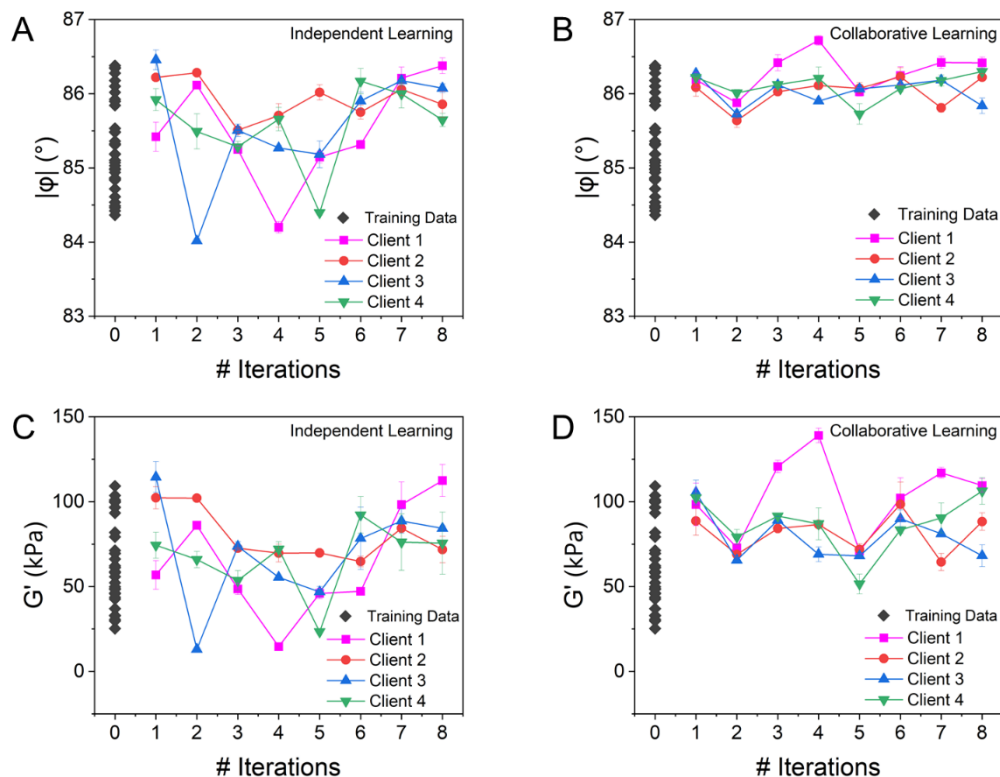


**Figure 5.5** Absolute phase angle  $|\varphi|$  (A, B) and  $G'$  (C, D) associated with distributed AMD driven by collaborative learning via consensus BO and traditional active learning by BO (i.e., without

collaboration). Labeled data acquired using brute-force HTE were used as the training data (i.e., iteration 0).

The results of the collaborative learning-driven AMD process over eight iterations in comparison with a control group in which the four clients did not collaborate are shown in Figure 5.5. Figure 5.5 displays the progress of collaborative workflow in terms of the cantilever rheometer raw data and the associated  $G'$ . As shown in Figure 5.5 B and D, collaboration among four clients working toward the shared material design objective achieved superior performance relative to the non-collaborative clients within six iterations (see Figure 5.5 A and C). For example, the formulations identified for the four collaborating clients across iterations 6 – 8 exhibited an average absolute phase angle value of  $86.17 \pm 0.18$  degrees and an average  $G'$  of  $91.56 \pm 15.32$  kPa. Additionally, the average difference between the average phase angle value and  $G'$  among successive iterations throughout iterations 6 – 8 were  $0.25 \pm 0.18$  degrees and  $27.11 \pm 15.32$  kPa, respectively. In contrast, the average absolute phase angle value and  $G'$  across iterations 6 – 8 in the control group composed of four non-collaborating clients were  $85.96 \pm 0.28$  degrees and  $81.16 \pm 15.92$  kPa, respectively. Likewise, the average difference between the average phase angle value and  $G'$  among successive iterations throughout iterations 6 – 8 were  $0.42 \pm 0.28$  degrees and  $33.97 \pm 15.92$  kPa, respectively. Furthermore, Figure 5.5 demonstrates that the collaborative clients exhibit a more consistent trend in phase angle and  $G'$  compared to the non-collaborative clients. For instance, the data in Figure 5.5 B (obtained with collaboration) exhibited a smaller variance than those shown in Figure 5.5 A and also exhibited a higher mean  $G'$ . In conclusion, the higher average  $G'$  obtained by clients in the final budgeted iterations, smaller average difference among successive final iterations, and the reduced variance among collaborating clients are indicative of AMD scalability and improved performance offered by collaborative learning. Given the time

required for each iteration was the same in both groups (i.e., the collaborating and non-collaborating groups of clients), the collaborative learning method increased the efficiency of the scaled AMD workflow.

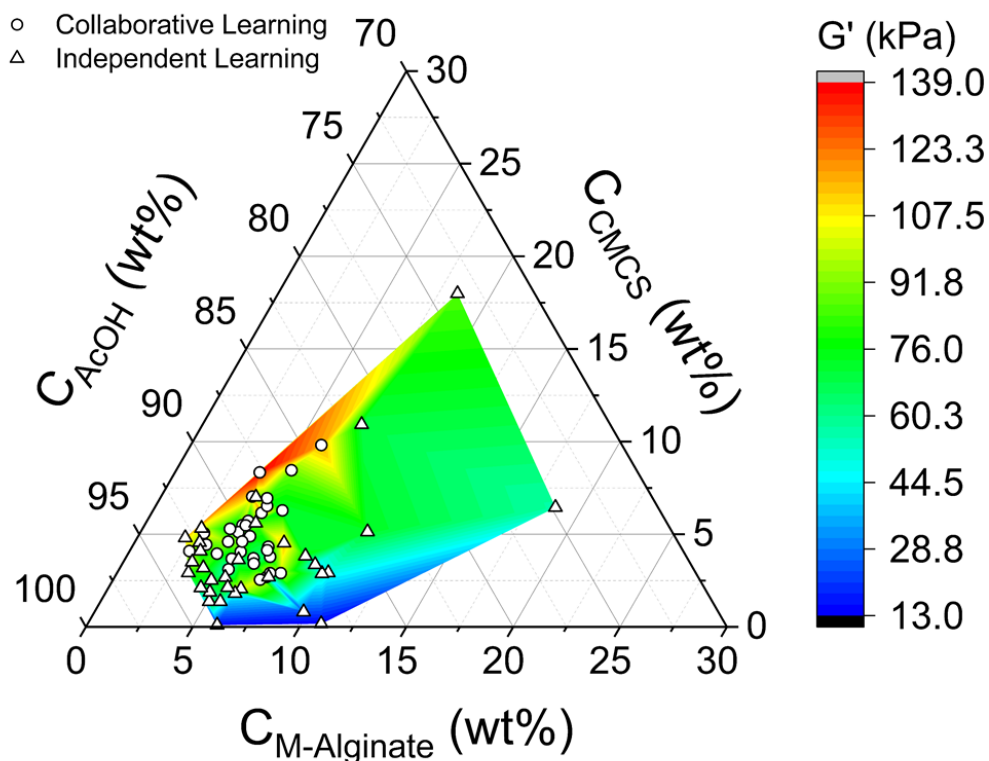


**Figure 5.6** Ternary diagram of the relationship between absolute phase angle and the composition of hydrogel.

The trends in formulation optimization visualized as a composition-property relation achieved by scaling the AMD process across four collaborating clients using consensus BO are highlighted in Figure 5.6 in comparison with traditional active learning using BO. The DIW content of all samples is provided as Supplementary Material (see Table SC - 2). The ternary diagram highlights the distribution of labeled data generated by the collaborative and non-collaborative learning models throughout their search for an optimal solution (i.e., formulation that

generates optimized hydrogel  $G'$ ). The collaborative learning model incentivized the collaborating clients to focus exploration in the design space where  $G'$  was relatively high, while the non-collaborative model drove the non-interacting clients to explore a relatively larger region of the design space where  $G'$  exhibited a wide range.

These results are expected based on the design and structure of the consensus BO model. For example, the consensus matrix (Equations (5) and (6)) is designed to support clients during the early stages when they lack sufficient data to fit high-quality surrogates, allowing them to leverage information from each other. In the later stages (i.e., iterations), we reduced the influence of other clients (i.e., shrink off-diagonal elements in the consensus matrix) to enable clients to focus on their individual experiments and obtain client-specific optimal formulations. As indicated by the distribution of brown points in Figure 5.6, collaborating clients working toward a shared AMD objective assisted each other in focusing on regions characterized by high  $G'$  (i.e., large absolute phase angle at resonance considering the output of the characterization tool), which was where most testing was recommended. In contrast, the non-collaborative group of clients expended more effort in regions associated with compositions exhibiting non-optimal  $G'$  (indicated by the green points in Figure 5.6).

## 5.4 Conclusions

In this study, we addressed a grand challenge in AMD, that of scalability. While brute-force HTE can accelerate material discovery and optimization tasks through process automation, the designer or manufacturer lacks certainty (e.g., quantitative assurance) that the identified formulation is optimal, given the high dimensionality of the design space. We first demonstrated that AMD workflows can be scaled by incorporating HTE with active learning and distributing effort across multiple AMD systems (i.e., clients) working toward a shared material design-optimization

objective. Importantly, we show that collaborative learning via consensus BO, which is based on collaboration among multiple clients, can establish a scalable closed-loop AMD workflow that outperforms that driven by traditional active learning via BO. The methodology was applied to AMD of composite glycogels that exhibit optimal mechanical properties, specifically  $G'$ . This work demonstrates the capability of using consensus BO to scale autonomous closed-loop AMD workflows across multiple systems composed of humans, machines, and combinations thereof.

## **6 Conclusions and Outlook**

This dissertation aims at accelerating materials discovery by enriching the toolbox of high-throughput characterization for soft materials. Sensor tools were used to investigate soft materials, and sensor-based high-throughput characterization was developed to optimize the formulation of composite hydrogels. Accelerated engineering of hydrogels was achieved via combining the sensor-based high-throughput experimentation and collaborative learning. This chapter will summarize the conclusions and contributions of the dissertation and discuss some potential future work.

### **6.1 Research contributions and conclusions**

The main contribution of this dissertation is applying sensors to investigate soft materials and developed a sensor-based high-throughput characterization for accelerated engineering of hydrogels. Specifically, the main contributions of this dissertation are as follows.

#### **(1) Application of embedded sensors for real-time investigation of the process-structure-rheological property of hydrogels**

As shown in Chapter 3, PEMC sensors were used for in-situ and real-time monitoring of the polymerization, gelation, and stimuli-response of hydrogels in a low-volume format. Specifically, the hydrogels prepared via bulk method and layer-by-layer method were compared which demonstrated the capability of the PEMC sensors to detect the subtle differences caused by the manufacturing process. While 3D and 4D printing have attracted much attention nowadays, there is not much research to study how the manufacturing process affects the structure and property of hydrogels in a real-time manner. Therefore, the dissertation provides a good tool for real-time investigating the process-structure-property of soft materials with a small volume.

#### **(2) Development of sensor-based high-throughput characterization of hydrogels**

Based on the research in Chapter 3, a sensor-based high-throughput characterization method was developed in Chapter 4. PEMC sensors together with portable electrodes were integrated with robots for automated high-throughput characterization of composite conductive hydrogels to find the optimized formulation with the best balance of mechanical and electrical properties. The high-throughput characterization of soft materials is a big challenge for MGI. While there are a few reports of using dispensing/extrusion systems to formulate hydrogels in a well-plate format, limited tools are available for their characterizations. The dissertation demonstrated the feasibility of integrating portable sensors with robots for high-throughput characterization of hydrogels in a well-plate format. And multi-property characterization is also possible when more than one sensor is used.

### **(3) Combination of collaborative learning and sensor-based high-throughput experimentation for accelerate engineering of hydrogels**

In Chapter 5, the sensor-based high-throughput experimentation was combined with collaborative learning to accelerate materials discovery. Under the guidance of collaborative learning, it demonstrated that the sensor-based high-throughput experimentation can optimize the formulation of hydrogels with fewer iterations. Although high-throughput computation has been applied to many materials, either hard or soft materials, it's not common to use machine learning directly to guide the experiments of soft materials. Specifically, current machine learning for materials discovery mainly focuses on one client, but the collaborative learning provides an effective method for multiple clients which provides a solution for information sharing to further accelerate materials discovery. Basically, the dissertation provided a good example of how designed machine learning algorithms can guide high-throughput experimentation to accelerate discovery of soft materials.

## 6.2 Outlook

Despite some contributions made in this dissertation, there is still a lot of work to be done to accelerate the discovery of soft materials. Currently, the sensor-based high-throughput characterization utilizes robots to move one sensor to characterize multiple samples orderly, but it has some drawbacks. For example, some residues may adhere to the sensor if the soft materials are sticky which would affect the accuracy of the measurement. Another problem is the path of the movement may affect the results because the evaporation of solvent is largely determined by the order of the test in a well-plate format. Therefore, multiple sensors may be used simultaneously to shorten the test time and to reduce cross-contamination. Another possible solution is developing sensor arrays, which seems possible due to the portable size of PEMC sensors. In this case, either well-plates or other containers may be considered. Another big challenge of the current high-throughput experimentation is multi-property characterization. Although two different sensors were used in this dissertation, it's not enough for practical applications. More properties should be characterized, and more tools should be developed. But the compatibility of different measurement formats would be a challenge. In addition, other aspects can also be improved. For example, the soft materials used for high-throughput experimentation should be expanded, the machine learning algorithms can also be further improved, and so on.

## Appendix A: Supplementary Materials for: Comparison of Bulk- vs. Layer-by-layer-cured Stimuli-responsive PNIPAM-Alginate Hydrogel Dynamic Viscoelastic Property Response via Embedded Sensors

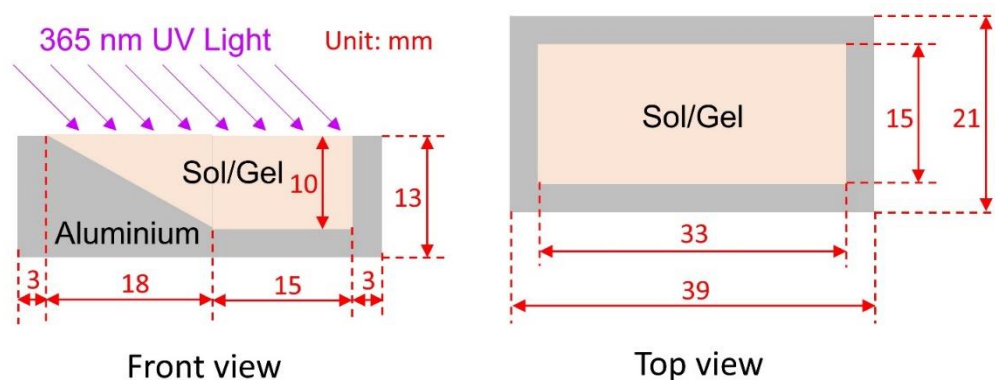


**Figure SA - 1** Schematic of the custom mold used for material gelation in this study.

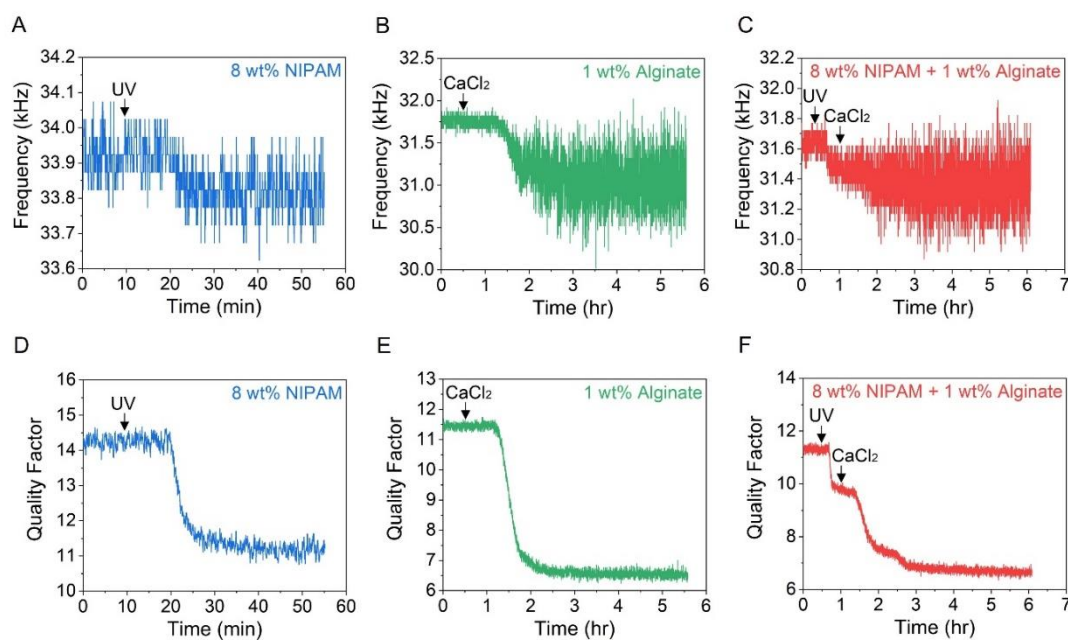


**Figure SA - 2** Real-time monitoring of resonant frequency (A, B, C) and quality factor (D, E, F) throughout gelation of PNIPAM, alginate, and PNIPAM-alginate composite hydrogels, respectively.

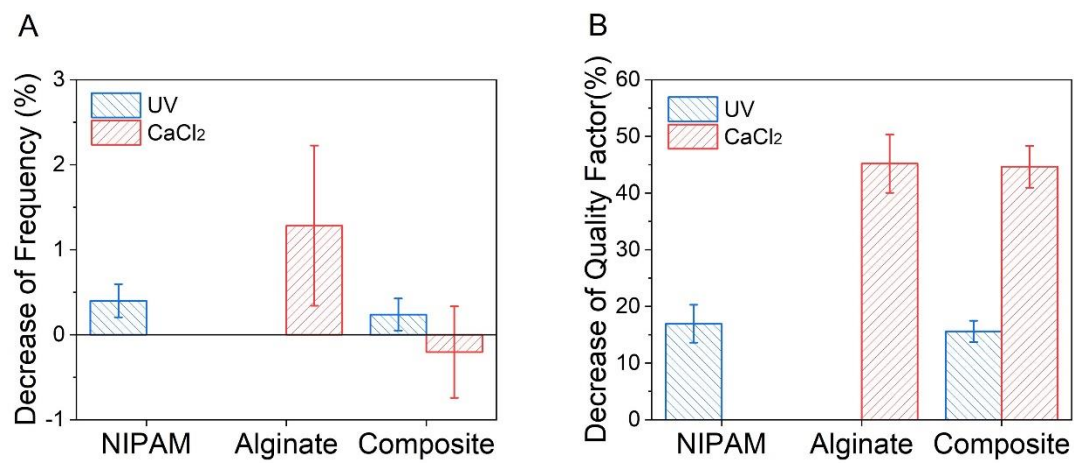


**Figure SA - 3** Comparison of the shift of resonant frequency (A) and quality factor (B) at each step of gelation process.

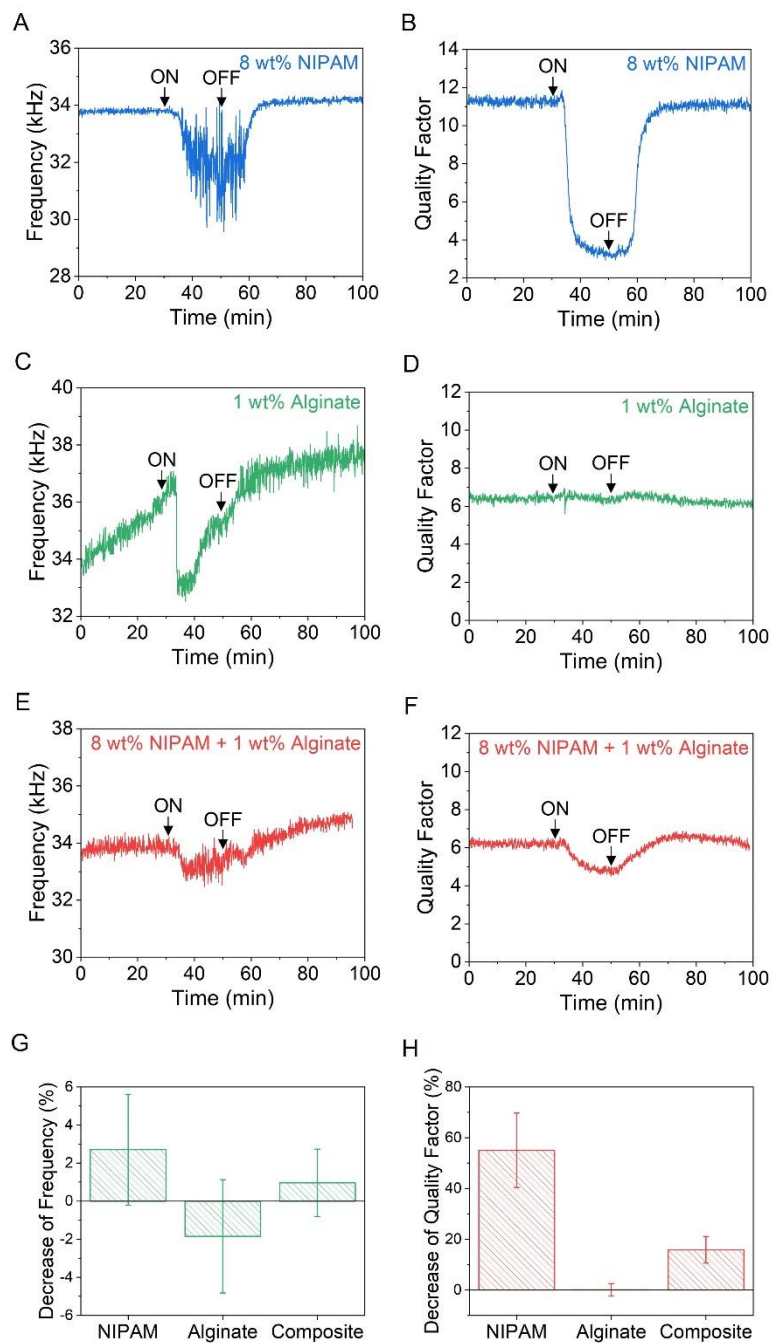


**Figure SA - 4** Real-time monitoring of resonant frequency (A, C, E) and quality factor (B, D, F) throughout a heating-cooling cycle of PNIPAM, alginate, and composite PNIPAM-alginate hydrogel. Their shifts of frequency (G) and quality factor (H) are also summarized and compared.

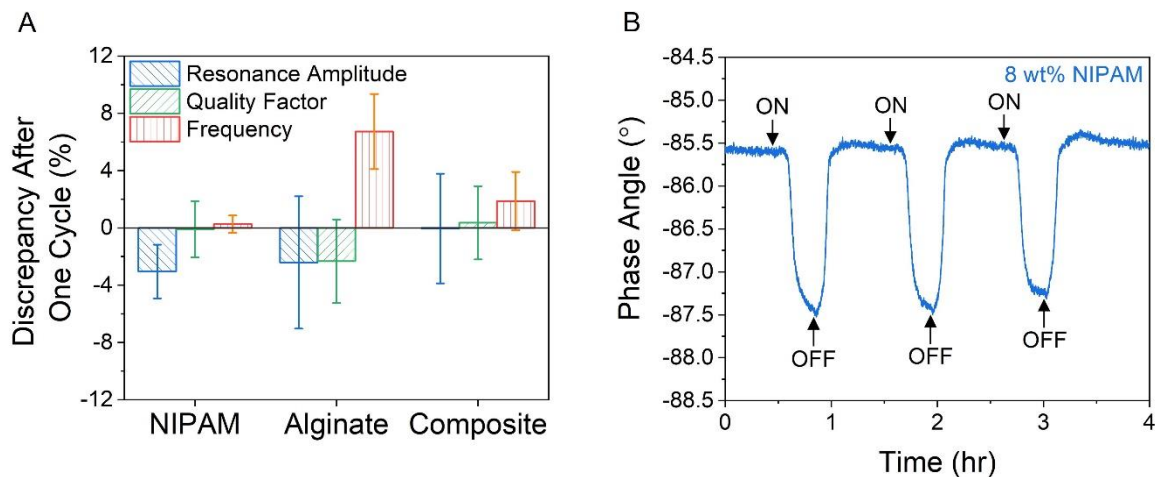


**Figure SA - 5** A) Deviations of resonance amplitude, resonant frequency, and quality factor after one heating-cooling cycle. B) Representative data associated with real-time monitoring of three heating-cooling cycles of PNIPAM hydrogels.

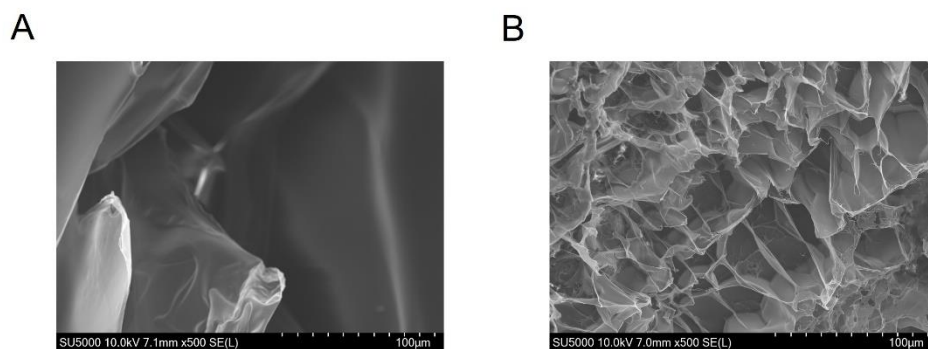


**Figure SA - 6** Comparison of PNIPAM hydrogel network structure below (A) and above (B) LCST.

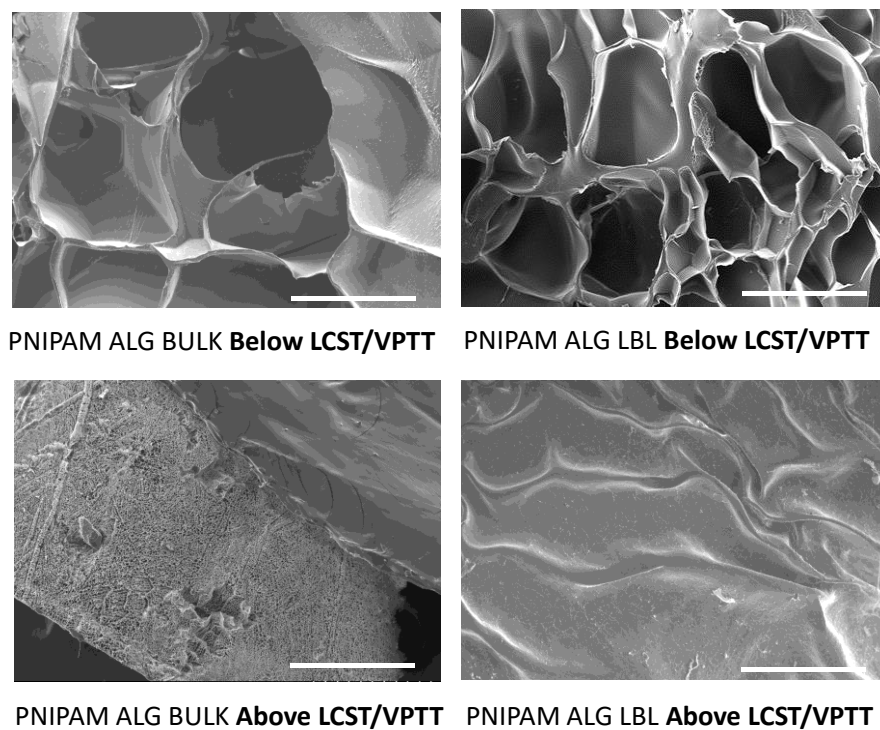


**Figure SA - 7** SEM of bulk- (A, C) and layer-by-layer (LBL)-cured (B, D) composite PNIPAM-alginate hydrogels below (A, B) and above (C, D) the VPTT of PNIPAM (scale bar = 500  $\mu\text{m}$ ).

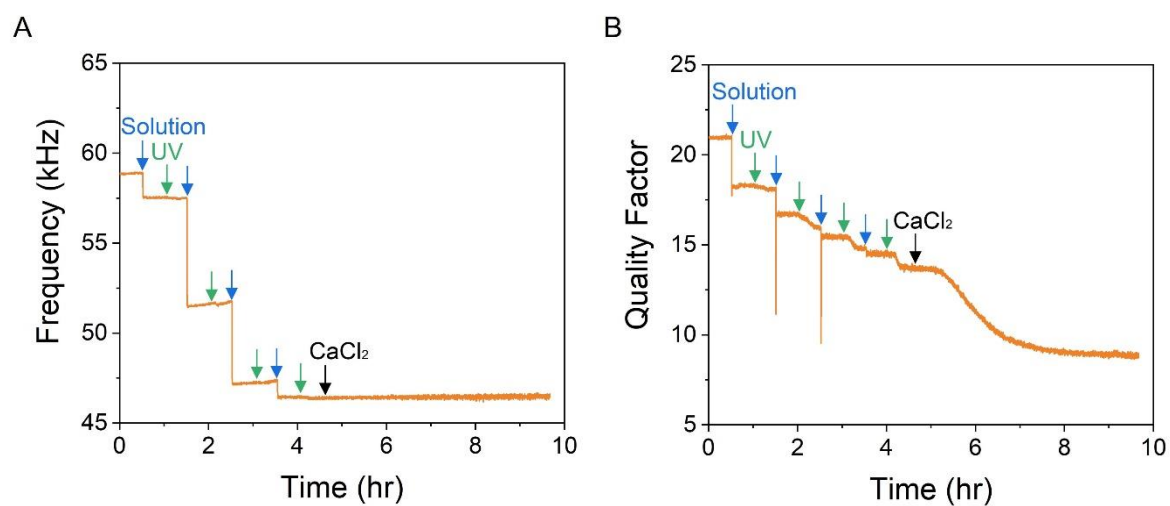


**Figure SA - 8** Real-time monitoring of LBL PNIPAM-alginate hydrogel construct fabrication via continuous tracking of resonant frequency (A) and quality factor (B).

## Appendix B: Supplementary Materials for: Accelerated Engineering of Optimized Functional Composite Hydrogels via High-Throughput Experimentation

### 1. Fluid-structure Interaction Model for Dynamic-Mode Cantilever Sensors

The shear moduli of the surrounding material (e.g., hydrogel) at the sensor's resonant frequency were calculated using the measured resonant frequency and quality factor, which is correlated with the phase angle at resonance, based on a fluid-structure interaction model established by Mather et al.<sup>150</sup>

$$g_1 = \frac{\pi}{2} \frac{b_2 G''}{\omega} + \frac{\pi}{4\sqrt{2}} \sqrt{\rho b} \left[ (b_1 - a_2) \sqrt{\sqrt{G'^2 + G''^2} + G'} + (a_2 + b_1) \sqrt{\sqrt{G'^2 + G''^2} - G'} \right] \quad (1)$$

$$g_2 = \frac{\pi}{4} a_1 \rho b^2 + \frac{\pi}{2} b_2 \frac{G'}{\omega^2} + \frac{\pi}{4\sqrt{2}} \frac{\sqrt{\rho b}}{\omega} \left[ (b_1 + a_2) \sqrt{\sqrt{G'^2 + G''^2} + G'} + (a_2 - b_1) \sqrt{\sqrt{G'^2 + G''^2} - G'} \right] \quad (2)$$

$$g_1 = \frac{\pi}{4} \rho b^2 \omega \left( \frac{(m_c + m_A) \omega}{Q} - c_i \right) \quad (3)$$

$$g_2 = \frac{\pi}{4} \rho b^2 \left( \frac{4\mu \left( \frac{\omega_0^2}{\omega^2} - 1 \right)}{\pi b^2 \rho} \right) \quad (4)$$

where  $L$  is the cantilever length,  $\mu = \rho_c b t$  is the cantilever mass per unit length,  $\rho_c$  and  $t$  are the respective cantilever density and thickness,  $Q_0$  and  $\omega_o$  are the respective quality factor and resonant frequency in the absence of fluid damping (i.e., resonating in vacuum with only internal damping effects present),  $m_c = \rho_c b t L$  is the cantilever mass,  $m_A = \rho \pi b^2 L \Gamma' / 4$  is the added mass,  $\Gamma'$  is the real part of the hydrodynamic function, and  $c_i = m_c \omega_o / Q_0$  is the internal damping coefficient. Due to the scale of the cantilevers ( $L = 4.1\text{mm}$ ) the internal damping was not negligible and was subtracted from the measured value (as described in the term  $c_i$  in Equation 3). In calculation of  $c_i$ ,  $\omega_o$  and

$Q_0$  were approximated as  $\omega_o \sim 2\pi f_{n,air}$  and  $Q_0 \sim Q_{n,air}$ , which were reasonable assumptions as discussed in the following sections. The hydrodynamic function was approximated using the relation  $\Gamma' = a_1 + a_2\delta/b$ , where  $\delta = [(2\eta/(\rho\omega))]^{1/2}$  is the thickness of the thin viscous layer surrounding the cantilever in which the velocity has dropped by a factor of  $1/e$ , and  $\eta$  is the viscosity of the fluid.

## 2. Additional Supplementary Material

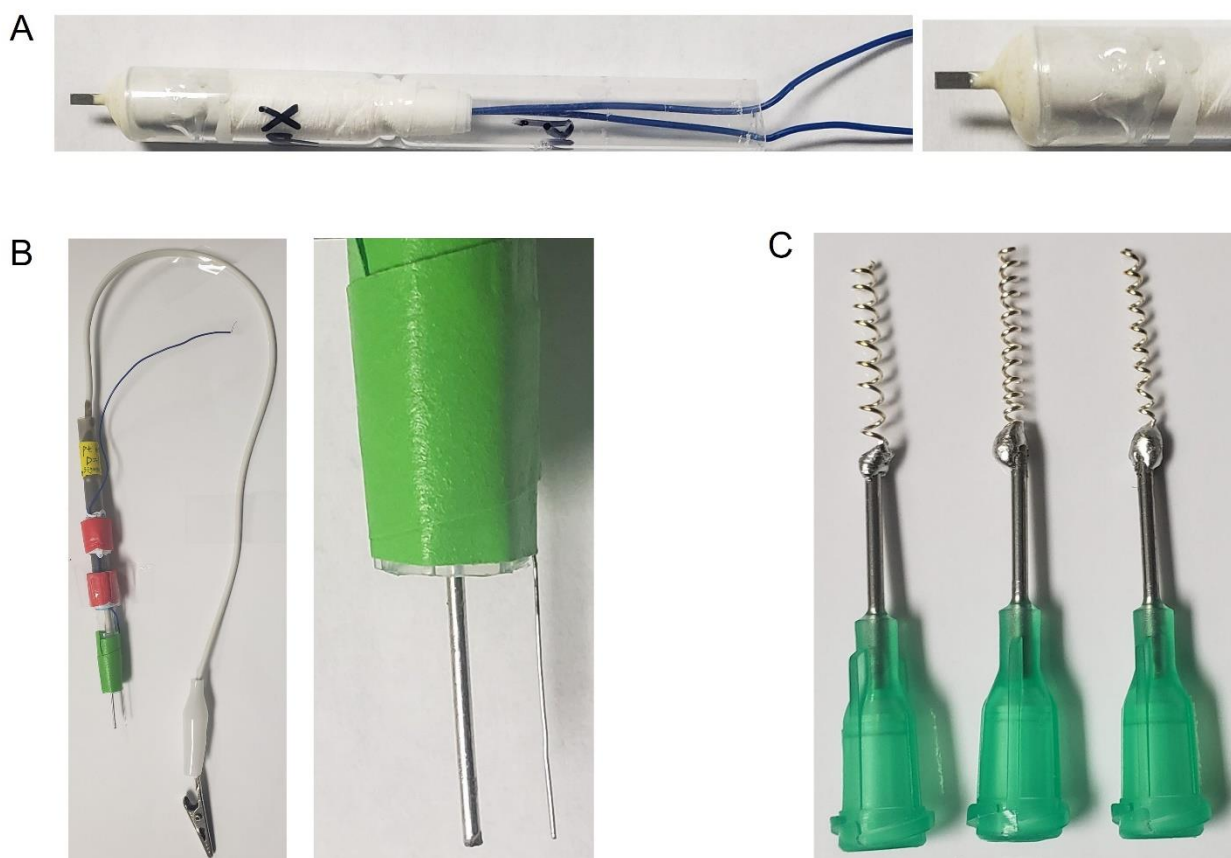


**Figure SB - 1** Photographs of piezoelectric milli-cantilever (PEMC) sensor (A), two-electrode setup used for characterization of electrical impedance (B), and custom helical stirring tools with diameters of 2.0, 1.7, and 1.4 mm (left to right, respectively).

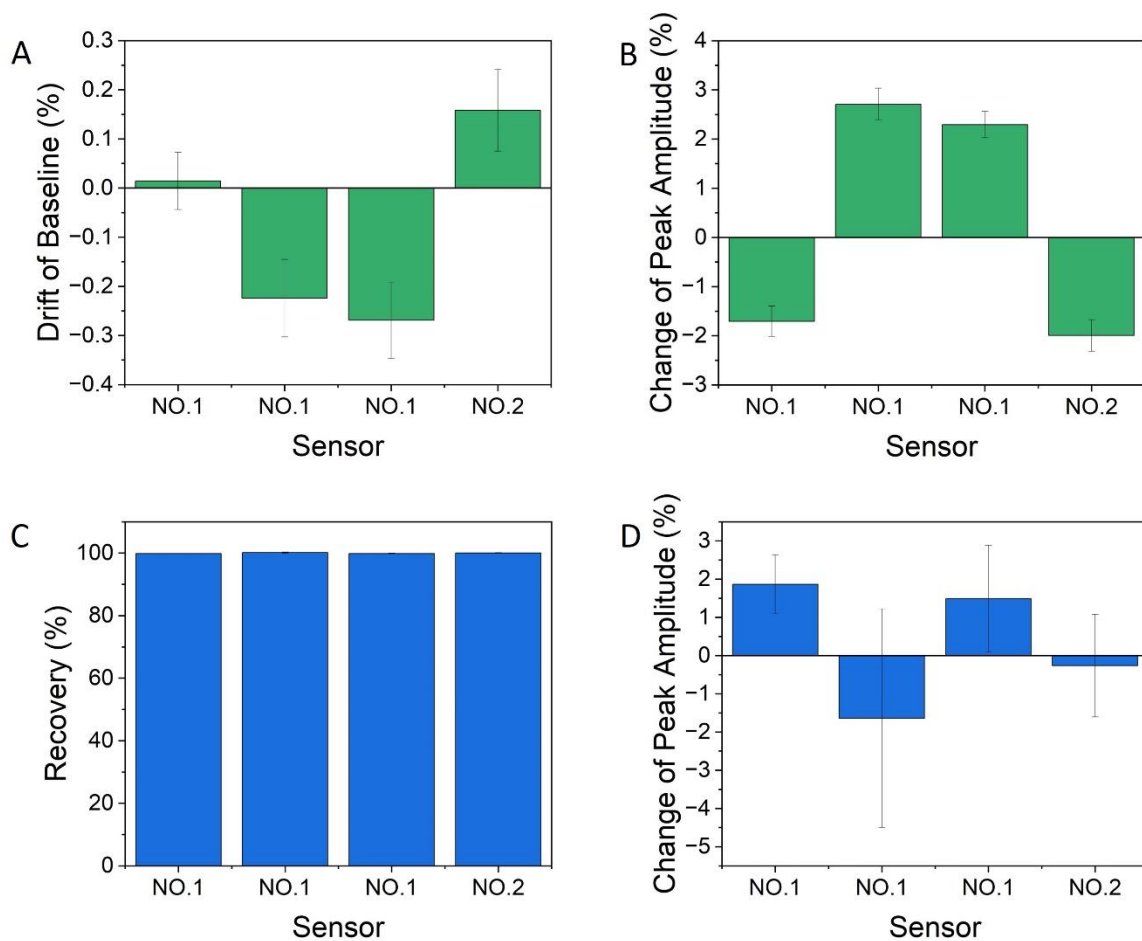


**Figure SB - 2** Analysis of sensor baseline response (A) and the percent change of the peak amplitude (B) of PEMC sensors after HTC of PEGDMA hydrogel libraries. Recovery of PEMC sensor phase angle (C), and the percent change of peak amplitude (D) relative to the baseline after removal from the hydrogels.

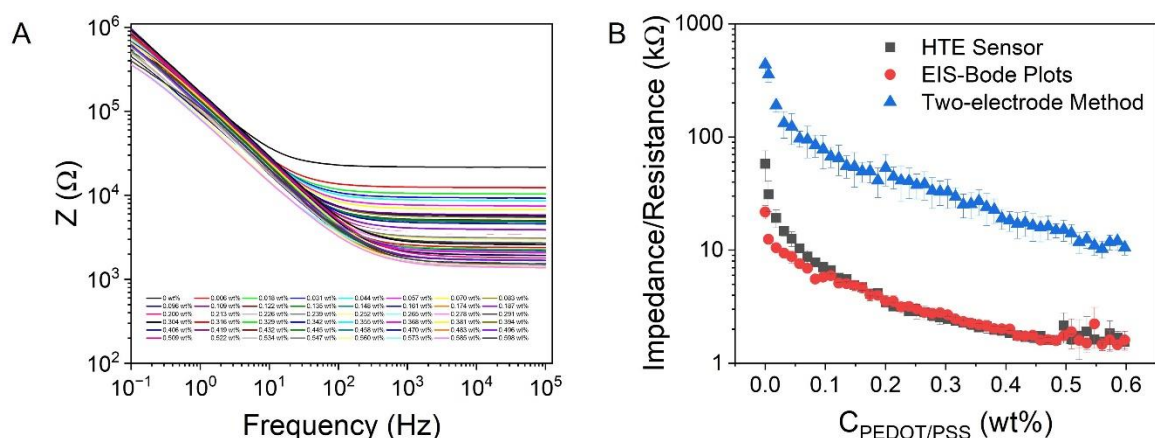


**Figure SB - 3** A) Bode plots of 48 PEGDMA/PEDOT:PSS hydrogels measured by conventional potentiostatic EIS. B) Impedance obtained by homemade impedance sensor and conventional potentiostatic EIS, and resistance obtained through two-electrode method. Impedance measurements were done in the well plate *in-situ*, while samples for resistance test were made via custom molds (40×4×3 mm3).

**Table SB - 1** The viscosities of solutions used in this research.

| Solution                                  | Viscosity ( $mPa \cdot S$ ) |
|-------------------------------------------|-----------------------------|
| Alginate (2 wt%)                          | 977.9                       |
| Alginate (0.5 wt%)                        | 35.1                        |
| Alginate (0.5 wt%) / NIPAM (4.57 wt%)     | 40.5                        |
| PEGDMA (25 wt%)                           | 11.9                        |
| PEGDMA (15 wt%)                           | 8.3                         |
| PEDOT : PSS (1.3 wt%)                     | 103.8                       |
| PEGDMA (13.1 wt%) / PEDOT : PSS (0.6 wt%) | 20.6                        |

Note: The viscosities were measured by ViscoQC 100 (Anton Paar) at room temperature.

## Appendix C: Supplementary Materials for: Scalable Accelerated Materials Discovery by Collaborative Learning

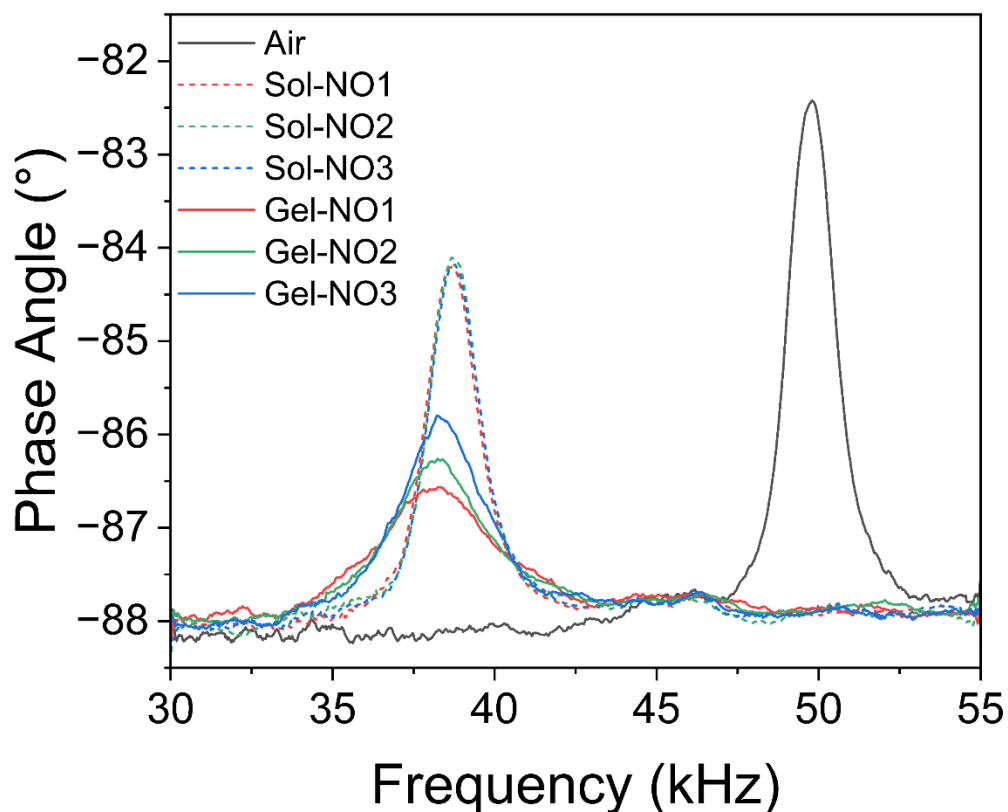


**Figure SC - 1** Spectra of the cantilever rheometer in three different M-alginate-CMCS mixtures (solutions) prior to AcOH addition and in the three resultant hydrogels illustrating the effect of mixture composition on hydrogel rheological properties ( $G'$ ). A reference spectrum in air is also shown to illustrate the characteristics of the cantilever rheometer.

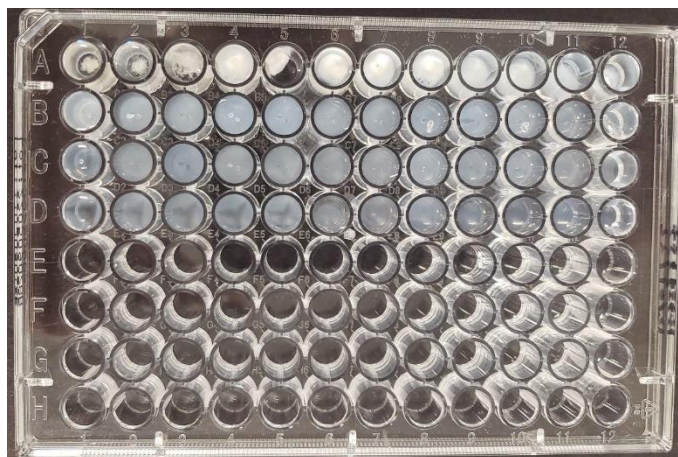


**Figure SC - 2** Photograph of formulated M-alginate-CMCS composite hydrogel samples illustrating phase separation at high CMCS concentrations (see main text for well number descriptions).

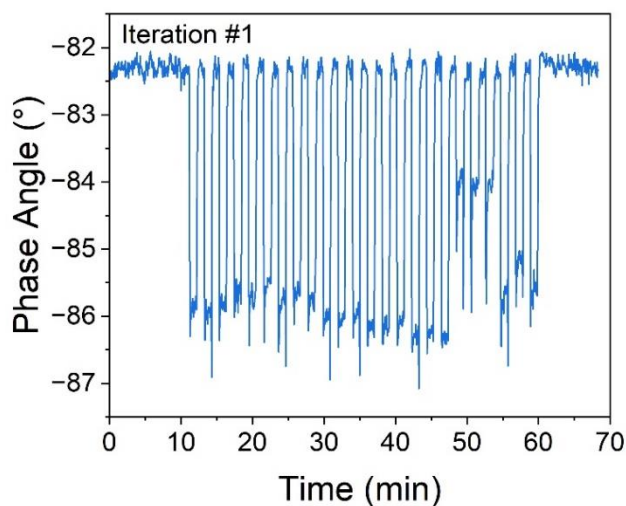


**Figure SC - 3** Representative time-series data obtained from the cantilever rheometer from the well plate-based automated high-throughput characterization format for a single experimental iteration. Samples from eight clients are tested per iteration (four collaborating clients and four non-collaborating clients), and each client examines three replicates.

**Table SC - 1** Composition of samples associated with the M-Alginate-CMCS composite hydrogels associated with the data shown in Figure 3 of the main text.

| Sample | Composition of All Components (wt%) |            |           |            | Composition Excluding Water (wt%) |            |            |
|--------|-------------------------------------|------------|-----------|------------|-----------------------------------|------------|------------|
|        | H <sub>2</sub> O                    | M-Alginate | CMCS      | AcOH       | M-Alginate                        | CMCS       | AcOH       |
| NO.1   | 99.13±0.00                          | 0.00±0.00  | 0.66±0.00 | 0.22±0.00  | 0.00±0.00                         | 75.14±0.26 | 24.86±0.26 |
| NO.2   | 99.32±0.00                          | 0.01±0.00  | 0.65±0.00 | 0.03±0.00  | 1.10±0.28                         | 95.09±0.32 | 3.81±0.08  |
| NO.3   | 98.35±0.02                          | 0.02±0.00  | 0.63±0.00 | 1.00±0.01  | 1.32±0.11                         | 38.40±0.35 | 60.28±0.34 |
| NO.4   | 90.66±0.08                          | 0.04±0.00  | 0.62±0.00 | 8.69±0.08  | 0.39±0.02                         | 6.64±0.05  | 92.97±0.06 |
| NO.5   | 99.32±0.00                          | 0.05±0.00  | 0.61±0.00 | 0.02±0.00  | 7.45±0.24                         | 89.78±0.30 | 2.77±0.12  |
| NO.6   | 99.28±0.00                          | 0.07±0.00  | 0.60±0.00 | 0.06±0.00  | 9.07±0.22                         | 83.18±0.23 | 7.75±0.07  |
| NO.7   | 93.21±0.07                          | 0.08±0.00  | 0.58±0.00 | 6.13±0.07  | 1.17±0.02                         | 8.55±0.11  | 90.29±0.11 |
| NO.8   | 99.26±0.00                          | 0.09±0.00  | 0.56±0.00 | 0.08±0.00  | 12.56±0.19                        | 76.06±0.24 | 11.37±0.20 |
| NO.9   | 90.09±0.08                          | 0.11±0.00  | 0.55±0.00 | 9.25±0.08  | 1.09±0.02                         | 5.57±0.04  | 93.34±0.05 |
| NO.10  | 98.87±0.00                          | 0.12±0.00  | 0.53±0.00 | 0.48±0.00  | 10.71±0.12                        | 47.12±0.14 | 42.16±0.17 |
| NO.11  | 95.28±0.09                          | 0.14±0.00  | 0.52±0.00 | 4.06±0.09  | 2.89±0.05                         | 11.09±0.24 | 86.01±0.28 |
| NO.12  | 97.87±0.12                          | 0.15±0.00  | 0.51±0.00 | 1.47±0.12  | 7.13±0.39                         | 24.04±1.47 | 68.83±1.86 |
| NO.13  | 98.42±0.01                          | 0.17±0.00  | 0.50±0.00 | 0.92±0.01  | 10.49±0.09                        | 31.40±0.26 | 58.11±0.32 |
| NO.14  | 99.30±0.00                          | 0.18±0.00  | 0.48±0.00 | 0.03±0.00  | 25.90±0.12                        | 69.27±0.14 | 4.83±0.05  |
| NO.15  | 99.28±0.00                          | 0.19±0.00  | 0.46±0.00 | 0.07±0.00  | 26.57±0.11                        | 63.83±0.15 | 9.60±0.14  |
| NO.16  | 93.69±0.08                          | 0.21±0.00  | 0.45±0.00 | 5.65±0.08  | 3.32±0.04                         | 7.20±0.10  | 89.49±0.14 |
| NO.17  | 88.46±0.10                          | 0.22±0.00  | 0.44±0.00 | 10.88±0.10 | 1.94±0.02                         | 3.81±0.03  | 94.25±0.04 |
| NO.18  | 99.28±0.00                          | 0.24±0.00  | 0.42±0.00 | 0.06±0.00  | 32.79±0.07                        | 58.52±0.11 | 8.68±0.10  |
| NO.19  | 99.30±0.00                          | 0.25±0.00  | 0.41±0.00 | 0.04±0.00  | 35.87±0.06                        | 58.31±0.08 | 5.82±0.02  |
| NO.20  | 98.38±0.13                          | 0.27±0.00  | 0.40±0.00 | 0.96±0.13  | 16.65±1.38                        | 24.71±2.10 | 58.63±3.48 |
| NO.21  | 88.00±0.10                          | 0.28±0.00  | 0.38±0.00 | 11.34±0.10 | 2.34±0.02                         | 3.17±0.02  | 94.50±0.04 |
| NO.22  | 99.20±0.01                          | 0.30±0.00  | 0.37±0.00 | 0.13±0.01  | 37.09±0.28                        | 46.04±0.27 | 16.87±0.54 |
| NO.23  | 99.27±0.00                          | 0.31±0.00  | 0.35±0.00 | 0.08±0.00  | 41.88±0.06                        | 47.62±0.12 | 10.50±0.17 |
| NO.24  | 95.78±0.09                          | 0.32±0.00  | 0.34±0.00 | 3.56±0.09  | 7.69±0.18                         | 8.02±0.20  | 84.29±0.38 |

|       |            |           |           |            |            |            |            |
|-------|------------|-----------|-----------|------------|------------|------------|------------|
| NO.25 | 89.50±0.09 | 0.34±0.00 | 0.32±0.00 | 9.83±0.09  | 3.23±0.02  | 3.09±0.02  | 93.68±0.05 |
| NO.26 | 99.30±0.01 | 0.35±0.00 | 0.31±0.00 | 0.05±0.01  | 49.77±0.51 | 43.58±0.41 | 6.65±0.92  |
| NO.27 | 94.73±0.08 | 0.37±0.00 | 0.30±0.00 | 4.61±0.08  | 6.99±0.12  | 5.60±0.10  | 87.41±0.22 |
| NO.28 | 99.34±0.00 | 0.38±0.00 | 0.28±0.00 | 0.00±0.00  | 57.31±0.17 | 42.06±0.06 | 0.63±0.22  |
| NO.29 | 98.78±0.00 | 0.39±0.00 | 0.26±0.00 | 0.56±0.00  | 32.15±0.10 | 21.57±0.11 | 46.28±0.21 |
| NO.30 | 98.50±0.01 | 0.41±0.00 | 0.25±0.00 | 0.83±0.01  | 27.49±0.17 | 16.83±0.13 | 55.68±0.30 |
| NO.31 | 99.04±0.00 | 0.42±0.00 | 0.23±0.00 | 0.31±0.00  | 43.78±0.07 | 24.41±0.10 | 31.81±0.15 |
| NO.32 | 89.04±0.09 | 0.44±0.00 | 0.22±0.00 | 10.30±0.09 | 4.00±0.02  | 2.02±0.02  | 93.98±0.04 |
| NO.33 | 97.36±0.11 | 0.45±0.00 | 0.21±0.00 | 1.98±0.11  | 17.13±0.79 | 7.86±0.34  | 75.01±1.13 |
| NO.34 | 92.64±0.07 | 0.47±0.00 | 0.19±0.00 | 6.70±0.07  | 6.38±0.07  | 2.64±0.03  | 90.99±0.09 |
| NO.35 | 99.33±0.00 | 0.48±0.00 | 0.18±0.00 | 0.01±0.00  | 71.67±0.22 | 26.62±0.11 | 1.71±0.17  |
| NO.36 | 98.61±0.01 | 0.49±0.00 | 0.16±0.00 | 0.74±0.01  | 36.34±0.20 | 11.72±0.11 | 52.95±0.28 |
| NO.37 | 92.16±0.07 | 0.51±0.00 | 0.15±0.00 | 7.18±0.07  | 6.50±0.06  | 1.91±0.02  | 91.59±0.08 |
| NO.38 | 91.12±0.07 | 0.52±0.00 | 0.14±0.00 | 8.22±0.07  | 5.90±0.04  | 1.53±0.02  | 92.57±0.06 |
| NO.39 | 96.82±0.10 | 0.54±0.00 | 0.12±0.00 | 2.52±0.11  | 17.04±0.63 | 3.83±0.12  | 79.13±0.74 |
| NO.40 | 96.32±0.10 | 0.55±0.00 | 0.11±0.00 | 3.02±0.10  | 15.02±0.45 | 2.90±0.07  | 82.08±0.52 |
| NO.41 | 94.24±0.08 | 0.57±0.00 | 0.09±0.00 | 5.10±0.08  | 9.84±0.15  | 1.60±0.03  | 88.55±0.17 |
| NO.42 | 98.95±0.00 | 0.58±0.00 | 0.08±0.00 | 0.39±0.00  | 55.16±0.05 | 7.40±0.14  | 37.44±0.10 |
| NO.43 | 99.29±0.00 | 0.60±0.00 | 0.06±0.00 | 0.05±0.00  | 84.20±0.21 | 8.99±0.20  | 6.80±0.04  |
| NO.44 | 91.59±0.07 | 0.61±0.00 | 0.05±0.00 | 7.75±0.07  | 7.30±0.06  | 0.59±0.02  | 92.11±0.07 |
| NO.45 | 98.91±0.13 | 0.63±0.00 | 0.04±0.00 | 0.43±0.13  | 58.47±7.97 | 3.25±0.36  | 38.28±8.31 |
| NO.46 | 98.68±0.01 | 0.64±0.00 | 0.02±0.00 | 0.66±0.01  | 48.59±0.24 | 1.56±0.12  | 49.85±0.24 |
| NO.47 | 87.53±0.10 | 0.65±0.00 | 0.01±0.00 | 11.81±0.10 | 5.22±0.03  | 0.05±0.01  | 94.73±0.04 |
| NO.48 | 87.21±0.08 | 0.65±0.00 | 0.00±0.00 | 12.13±0.08 | 5.12±0.03  | 0.00±0.00  | 94.88±0.03 |

**Table SC - 2** Composition of samples associated with the M-Alginate-CMCS composite hydrogels associated with the data shown in Figure 6 of the main text.

|                      |           |        | Weight Percent of all Components (wt%) |            |      |       | Weight Percent Exclude Water (wt%) |       |       |
|----------------------|-----------|--------|----------------------------------------|------------|------|-------|------------------------------------|-------|-------|
|                      | Iteration | Client | H <sub>2</sub> O                       | M-Alginate | CMCS | AcOH  | M-Alginate                         | CMCS  | AcOH  |
| Independent Learning | 0         | 1      | 97.38                                  | 0.49       | 0.17 | 1.96  | 18.80                              | 6.45  | 74.80 |
|                      |           | 2      | 92.04                                  | 0.50       | 0.16 | 7.30  | 6.24                               | 2.06  | 91.71 |
|                      |           | 3      | 94.28                                  | 0.40       | 0.26 | 5.06  | 7.02                               | 4.53  | 88.46 |
|                      |           | 4      | 89.79                                  | 0.52       | 0.14 | 9.55  | 5.09                               | 1.37  | 93.54 |
|                      | 1         | 1      | 97.50                                  | 0.21       | 0.45 | 1.84  | 8.40                               | 18.00 | 73.61 |
|                      |           | 2      | 91.83                                  | 0.22       | 0.44 | 7.51  | 2.75                               | 5.32  | 91.93 |
|                      |           | 3      | 94.06                                  | 0.65       | 0.01 | 5.28  | 10.90                              | 0.17  | 88.90 |
|                      |           | 4      | 90.22                                  | 0.48       | 0.18 | 9.12  | 4.87                               | 1.88  | 93.25 |
|                      | 2         | 1      | 94.84                                  | 0.51       | 0.15 | 4.50  | 9.88                               | 2.92  | 87.20 |
|                      |           | 2      | 95.81                                  | 0.44       | 0.22 | 3.53  | 10.60                              | 5.13  | 84.25 |
|                      |           | 3      | 94.59                                  | 0.45       | 0.21 | 4.75  | 8.36                               | 3.83  | 87.81 |
|                      |           | 4      | 94.72                                  | 0.51       | 0.15 | 4.62  | 9.62                               | 2.88  | 87.5  |
|                      | 3         | 1      | 89.34                                  | 0.65       | 0.01 | 10.00 | 6.10                               | 0.09  | 93.81 |
|                      |           | 2      | 89.76                                  | 0.44       | 0.22 | 9.58  | 4.33                               | 2.12  | 93.55 |
|                      |           | 3      | 90.54                                  | 0.53       | 0.13 | 8.80  | 5.61                               | 1.37  | 93.02 |
|                      |           | 4      | 90.75                                  | 0.43       | 0.23 | 8.59  | 4.62                               | 2.52  | 92.86 |
|                      | 4         | 1      | 94.68                                  | 0.48       | 0.18 | 4.66  | 9.05                               | 3.35  | 87.60 |
|                      |           | 2      | 96.41                                  | 0.27       | 0.39 | 2.93  | 7.44                               | 10.90 | 81.63 |
|                      |           | 3      | 93.33                                  | 0.48       | 0.18 | 6.01  | 7.20                               | 2.70  | 90.10 |
|                      |           | 4      | 93.77                                  | 0.61       | 0.05 | 5.57  | 9.79                               | 0.81  | 89.40 |
|                      | 5         | 1      | 91.64                                  | 0.51       | 0.15 | 7.70  | 6.08                               | 1.82  | 92.11 |
|                      |           | 2      | 92.63                                  | 0.39       | 0.27 | 6.71  | 5.33                               | 3.63  | 91.04 |
|                      |           | 3      | 89.45                                  | 0.35       | 0.31 | 9.89  | 3.34                               | 2.92  | 93.74 |
|                      |           | 4      | 91.31                                  | 0.26       | 0.4  | 8.03  | 3.04                               | 4.55  | 92.40 |

|                        |   |   |       |      |      |      |      |      |       |
|------------------------|---|---|-------|------|------|------|------|------|-------|
| Collaborative Learning | 6 | 1 | 90.18 | 0.32 | 0.34 | 9.16 | 3.23 | 3.50 | 93.28 |
|                        |   | 2 | 90.69 | 0.36 | 0.30 | 8.65 | 3.9  | 3.19 | 92.91 |
|                        |   | 3 | 94.25 | 0.26 | 0.40 | 5.09 | 4.46 | 7.01 | 88.53 |
|                        |   | 4 | 91.09 | 0.30 | 0.36 | 8.25 | 3.32 | 4.09 | 92.59 |
|                        |   | 1 | 90.65 | 0.21 | 0.45 | 8.69 | 2.25 | 4.81 | 92.94 |
|                        |   | 2 | 91.61 | 0.44 | 0.22 | 7.73 | 5.19 | 2.68 | 92.14 |
|                        |   | 3 | 93.87 | 0.32 | 0.34 | 5.47 | 5.15 | 5.61 | 89.24 |
|                        |   | 4 | 91.43 | 0.48 | 0.18 | 7.91 | 5.58 | 2.13 | 92.29 |
|                        | 7 | 1 | 93.76 | 0.48 | 0.18 | 5.58 | 7.67 | 2.90 | 89.42 |
|                        |   | 2 | 93.28 | 0.48 | 0.18 | 6.06 | 7.12 | 2.70 | 90.18 |
|                        |   | 3 | 93.45 | 0.47 | 0.19 | 5.89 | 7.17 | 2.90 | 89.92 |
|                        |   | 4 | 93.00 | 0.48 | 0.18 | 6.34 | 6.88 | 2.55 | 90.57 |
|                        | 1 | 1 | 93.83 | 0.39 | 0.27 | 5.51 | 6.38 | 4.33 | 89.30 |
|                        |   | 2 | 93.73 | 0.42 | 0.24 | 5.61 | 6.73 | 3.79 | 89.48 |
|                        |   | 3 | 93.75 | 0.40 | 0.26 | 5.59 | 6.42 | 4.14 | 89.44 |
|                        |   | 4 | 93.48 | 0.34 | 0.32 | 5.86 | 5.23 | 4.90 | 89.87 |
|                        | 2 | 1 | 94.16 | 0.30 | 0.36 | 5.18 | 5.15 | 6.15 | 88.70 |
|                        |   | 2 | 92.88 | 0.37 | 0.29 | 6.46 | 5.21 | 4.06 | 90.73 |
|                        |   | 3 | 93.45 | 0.30 | 0.36 | 5.89 | 4.59 | 5.49 | 89.92 |
|                        |   | 4 | 92.63 | 0.32 | 0.34 | 6.71 | 4.36 | 4.60 | 91.04 |
|                        | 3 | 1 | 94.64 | 0.21 | 0.45 | 4.70 | 3.97 | 8.34 | 87.69 |
|                        |   | 2 | 93.19 | 0.31 | 0.35 | 6.15 | 4.58 | 5.11 | 90.30 |
|                        |   | 3 | 92.39 | 0.38 | 0.28 | 6.95 | 4.99 | 3.67 | 91.33 |
|                        |   | 4 | 93.14 | 0.34 | 0.32 | 6.20 | 5.01 | 4.61 | 90.38 |
|                        | 4 | 1 | 94.65 | 0.32 | 0.34 | 4.69 | 6.06 | 6.29 | 87.65 |
|                        |   | 2 | 92.97 | 0.29 | 0.37 | 6.37 | 4.10 | 5.29 | 90.61 |
|                        |   | 3 | 93.69 | 0.30 | 0.36 | 5.65 | 4.74 | 5.72 | 89.54 |
|                        |   | 4 | 91.98 | 0.41 | 0.25 | 7.36 | 5.12 | 3.11 | 91.77 |
|                        | 5 | 1 | 94.38 | 0.29 | 0.37 | 4.96 | 5.22 | 6.53 | 88.25 |

|  |   |   |       |      |      |      |      |      |       |
|--|---|---|-------|------|------|------|------|------|-------|
|  |   | 2 | 90.43 | 0.27 | 0.39 | 8.91 | 2.80 | 4.10 | 93.10 |
|  |   | 3 | 93.54 | 0.31 | 0.35 | 5.80 | 4.74 | 5.48 | 89.78 |
|  |   | 4 | 91.87 | 0.34 | 0.32 | 7.47 | 4.16 | 3.96 | 91.88 |
|  | 6 | 1 | 95.86 | 0.25 | 0.41 | 3.48 | 6.12 | 9.81 | 84.07 |
|  |   | 2 | 93.20 | 0.41 | 0.25 | 6.14 | 5.99 | 3.71 | 90.30 |
|  |   | 3 | 94.48 | 0.28 | 0.38 | 4.86 | 5.02 | 6.94 | 88.04 |
|  |   | 4 | 95.24 | 0.26 | 0.40 | 4.10 | 5.40 | 8.46 | 86.15 |
|  | 7 | 1 | 94.16 | 0.25 | 0.41 | 5.18 | 4.27 | 7.03 | 88.69 |
|  |   | 2 | 91.60 | 0.29 | 0.37 | 7.74 | 3.41 | 4.44 | 92.14 |
|  |   | 3 | 93.10 | 0.42 | 0.24 | 6.24 | 6.15 | 3.42 | 90.43 |
|  |   | 4 | 91.82 | 0.25 | 0.41 | 7.52 | 3.00 | 5.06 | 91.94 |

## References

- (1) Maine, E.; Garnsey, E. Commercializing generic technology: The case of advanced materials ventures. *Res. Policy* **2006**, 35 (3), 375-393.
- (2) McDowell, D. L.; Kalidindi, S. R. The materials innovation ecosystem: A key enabler for the Materials Genome Initiative. *MRS Bull.* **2016**, 41 (4), 326-337.
- (3) Green, M. L.; Choi, C. L.; Hattrick-Simpers, J. R.; Joshi, A. M.; Takeuchi, I.; Barron, S. C.; Campo, E.; Chiang, T.; Empedocles, S.; Gregoire, J. M.; et al. Fulfilling The Promise of The Materials Genome Initiative With High-Throughput Experimental Methodologies. *Appl. Phys. Rev.* **2017**, 4 (1), 18.
- (4) Callahan, L. A. S. Combinatorial Method/High Throughput Strategies for Hydrogel Optimization in Tissue Engineering Applications. *Gels* **2016**, 2 (2), 16.
- (5) Nosengo, N. Can artificial intelligence create the next wonder material? *Nature* **2016**, 533 (7601), 22-25.
- (6) Kennedy, K.; Stefansky, T.; Davy, G.; Zackay, V. F.; Parker, E. R. Rapid Method for Determining Ternary-Alloy Phase Diagrams. *J. Appl. Phys.* **1965**, 36 (12), 3808-3810.
- (7) Hanak, J. J. The “multiple-sample concept” in materials research: Synthesis, compositional analysis and testing of entire multicomponent systems. *J. Mater. Sci.* **1970**, 5 (11), 964-971.
- (8) Potyrailo, R.; Rajan, K.; Stoewe, K.; Takeuchi, I.; Chisholm, B.; Lam, H. Combinatorial and High-Throughput Screening of Materials Libraries: Review of State of the Art. *ACS Comb. Sci.* **2011**, 13 (6), 579-633.
- (9) Green, M. L.; Takeuchi, I.; Hattrick-Simpers, J. R. Applications of High Throughput (Combinatorial) Methodologies to Electronic, Magnetic, Optical, and Energy-Related Materials. *J. Appl. Phys.* **2013**, 113 (23), 53.

- (10) Yu, Z.; Huang, W. Accelerating Optimizing the Design of Carbon-based Electrocatalyst via Machine Learning. *Electroanalysis* **2022**, *34* (4), 599-607.
- (11) Cheng, B.; Griffiths, R.-R.; Wengert, S.; Kunkel, C.; Stenczel, T.; Zhu, B.; Deringer, V. L.; Bernstein, N.; Margraf, J. T.; Reuter, K.; et al. Mapping Materials and Molecules. *Acc. Chem. Res.* **2020**, *53* (9), 1981-1991.
- (12) Liu, Y.; Niu, C.; Wang, Z.; Gan, Y.; Zhu, Y.; Sun, S.; Shen, T. Machine learning in materials genome initiative: A review. *J. Mater. Res. Technol.* **2020**, *57*, 113-122.
- (13) Jablonka, K. M.; Ongari, D.; Moosavi, S. M.; Smit, B. Big-Data Science in Porous Materials: Materials Genomics and Machine Learning. *Chem. Rev.* **2020**, *120* (16), 8066-8129.
- (14) Gromski, P. S.; Henson, A. B.; Granda, J. M.; Cronin, L. How to explore chemical space using algorithms and automation. *Nat. Rev. Chem.* **2019**, *3* (2), 119-128.
- (15) Himanen, L.; Geurts, A.; Foster, A. S.; Rinke, P. Data-Driven Materials Science: Status, Challenges, and Perspectives. *Adv. Sci.* **2019**, *6* (21), 1900808.
- (16) Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. Combinatorial synthesis — the design of compound libraries and their application to drug discovery. *Tetrahedron* **1995**, *51* (30), 8135-8173.
- (17) Thompson, L. A.; Ellman, J. A. Synthesis and Applications of Small Molecule Libraries. *Chem. Rev.* **1996**, *96* (1), 555-600.
- (18) Potyrailo, R. A.; Mirsky, V. M. Combinatorial and High-Throughput Development of Sensing Materials: The First 10 Years. *Chem. Rev.* **2008**, *108* (2), 770-813.
- (19) Kuo, T.-C.; Malvadkar, N. A.; Drumright, R.; Cesaretti, R.; Bishop, M. T. High-Throughput Industrial Coatings Research at The Dow Chemical Company. *ACS Comb. Sci.* **2016**, *18* (9), 507-526.

- (20) Gomez, I. J.; Wu, J.; Roper, J.; Beckham, H.; Meredith, J. C. High Throughput Screening of Mechanical Properties and Scratch Resistance of Tricomponent Polyurethane Coatings. *ACS Appl. Polym. Mater.* **2019**, *1* (11), 3064-3073.
- (21) Liu, Y.; Hu, Z.; Suo, Z.; Hu, L.; Feng, L.; Gong, X.; Liu, Y.; Zhang, J. High-Throughput Experiments Facilitate Materials Innovation: A review. *Sci. China Technol. Sci.* **2019**, *62* (4), 521-545.
- (22) Müller, C. M.; Sologubenko, A. S.; Gerstl, S. S. A.; Süess, M. J.; Courty, D.; Spolenak, R. Nanoscale Cu/Ta multilayer deposition by co-sputtering on a rotating substrate. Empirical model and experiment. *Surf. Coat. Technol.* **2016**, *302*, 284-292.
- (23) Bahrami, A.; Álvarez, J. P.; Depablos-Rivera, O.; Mirabal-Rojas, R.; Ruíz-Ramírez, A.; Muhl, S.; Rodil, S. E. Compositional and Tribo-Mechanical Characterization of Ti-Ta Coatings Prepared by Confocal Dual Magnetron Co-Sputtering. *Adv. Eng. Mater.* **2018**, *20* (3), 1700687.
- (24) Wang, X.; Rogalla, D.; Ludwig, A. Influences of W Content on the Phase Transformation Properties and the Associated Stress Change in Thin Film Substrate Combinations Studied by Fabrication and Characterization of Thin Film  $V_{1-x}W_xO_2$  Materials Libraries. *ACS Comb. Sci.* **2018**, *20* (4), 229-236.
- (25) Motemani, Y.; Khare, C.; Savan, A.; Hans, M.; Paulsen, A.; Frenzel, J.; Somsen, C.; Mücklich, F.; Eggeler, G.; Ludwig, A. Nanostructured Ti-Ta thin films synthesized by combinatorial glancing angle sputter deposition. *Nanotechnology* **2016**, *27* (49), 495604.
- (26) Xing, H.; Zhao, B.; Wang, Y.; Zhang, X.; Ren, Y.; Yan, N.; Gao, T.; Li, J.; Zhang, L.; Wang, H. Rapid Construction of Fe-Co-Ni Composition-Phase Map by Combinatorial Materials Chip Approach. *ACS Comb. Sci.* **2018**, *20* (3), 127-131.

- (27) Decker, P.; Naujoks, D.; Langenkämper, D.; Somsen, C.; Ludwig, A. High-Throughput Structural and Functional Characterization of the Thin Film Materials System Ni–Co–Al. *ACS Comb. Sci.* **2017**, *19* (10), 618-624.
- (28) McGinn, P. J. Combinatorial electrochemistry – Processing and characterization for materials discovery. *Mater. Discov.* **2015**, *1*, 38-53.
- (29) Hofmann, D. C.; Roberts, S.; Otis, R.; Kolodziejska, J.; Dillon, R. P.; Suh, J.-o.; Shapiro, A. A.; Liu, Z.-K.; Borgonia, J.-P. Developing Gradient Metal Alloys through Radial Deposition Additive Manufacturing. *Sci. Rep.* **2014**, *4* (1), 5357.
- (30) Knoll, H.; Ocylok, S.; Weisheit, A.; Springer, H.; Jäggle, E.; Raabe, D. Combinatorial Alloy Design by Laser Additive Manufacturing. *Steel Res. Int.* **2017**, *88* (8), 1600416.
- (31) Zhao, J.-C.; Xu, Y.; Hartmann, U. Measurement of an Iso-Curie Temperature Line of a Co-Cr-Mo Solid Solution by Magnetic Force Microscopy Imaging on a Diffusion Multiple. *Adv. Eng. Mater.* **2013**, *15* (5), 321-324.
- (32) Zhao, J. C.; Zheng, X.; Cahill, D. G. High-throughput diffusion multiples. *Mater. Today* **2005**, *8* (10), 28-37.
- (33) Chen, P.-C.; Liu, X.; Hedrick, J. L.; Xie, Z.; Wang, S.; Lin, Q.-Y.; Hersam, M. C.; Dravid, V. P.; Mirkin, C. A. Polyelemental nanoparticle libraries. *Science* **2016**, *352* (6293), 1565-1569.
- (34) Fenton, J. L.; Steimle, B. C.; Schaak, R. E. Tunable intraparticle frameworks for creating complex heterostructured nanoparticle libraries. *Science* **2018**, *360* (6388), 513-517.
- (35) Chen, L.; Luo, A.; Zhang, Y.; Liu, F.; Jiang, Y.; Xu, Q.; Chen, X.; Hu, Q.; Chen, S.-F.; Chen, K.-J.; et al. Optimization of the Single-Phased White Phosphor of Li<sub>2</sub>SrSiO<sub>4</sub>: Eu<sup>2+</sup>, Ce<sup>3+</sup> for Light-Emitting Diodes by Using the Combinatorial Approach Assisted with the Taguchi Method. *ACS Comb. Sci.* **2012**, *14* (12), 636-644.

- (36) Haber, J. A.; Guevarra, D.; Jung, S.; Jin, J.; Gregoire, J. M. Discovery of New Oxygen Evolution Reaction Electrocatalysts by Combinatorial Investigation of the Ni–La–Co–Ce Oxide Composition Space. *ChemElectroChem* **2014**, *1* (10), 1613-1617.
- (37) Shinde, A.; Jones, R. J. R.; Guevarra, D.; Mitrovic, S.; Becerra-Stasiewicz, N.; Haber, J. A.; Jin, J.; Gregoire, J. M. High-Throughput Screening for Acid-Stable Oxygen Evolution Electrocatalysts in the (Mn–Co–Ta–Sb)O<sub>x</sub> Composition Space. *Electrocatalysis* **2015**, *6* (2), 229-236.
- (38) Moon, H.; Jeong, S. J.; Lee, Y. T.; Sohn, D. Preparation of a Water-based Al/Fe/Mo Catalyst Using a Microfluidic System. *Chem. Lett.* **2010**, *39* (8), 814-815.
- (39) Saito, K.; Ueoka, K.; Matsumoto, K.; Suga, S.; Nokami, T.; Yoshida, J.-i. Indirect Cation-Flow Method: Flash Generation of Alkoxycarbenium Ions and Studies on the Stability of Glycosyl Cations. *Angew. Chem. Int. Ed.* **2011**, *50* (22), 5153-5156.
- (40) Gütz, C.; Klöckner, B.; Waldvogel, S. R. Electrochemical Screening for Electroorganic Synthesis. *Org. Process Res. Dev.* **2016**, *20* (1), 26-32.
- (41) Welch, A. W.; Baranowski, L. L.; Zawadzki, P.; Lany, S.; Wolden, C. A.; Zakutayev, A. CuSbSe<sub>2</sub> photovoltaic devices with 3% efficiency. *Appl. Phys. Express* **2015**, *8* (8), 082301.
- (42) Zhou, L.; Yan, Q.; Shinde, A.; Guevarra, D.; Newhouse, P. F.; Becerra-Stasiewicz, N.; Chatman, S. M.; Haber, J. A.; Neaton, J. B.; Gregoire, J. M. High Throughput Discovery of Solar Fuels Photoanodes in the CuO–V<sub>2</sub>O<sub>5</sub> System. *Adv. Energy Mater.* **2015**, *5* (22), 1500968.
- (43) Gregoire, J. M.; Van Campen, D. G.; Miller, C. E.; Jones, R. J. R.; Suram, S. K.; Mehta, A. High-Throughput Synchrotron X-ray Diffraction for Combinatorial Phase Mapping. *J. Synchrotron Radiat.* **2014**, *21*, 1262-1268.

- (44) Newbury, D. E.; Ritchie, N. W. M. Elemental mapping of microstructures by scanning electron microscopy-energy dispersive X-ray spectrometry (SEM-EDS): extraordinary advances with the silicon drift detector (SDD). *J. Anal. At. Spectrom.* **2013**, *28* (7), 973-988.
- (45) Thuriot-Roukos, J.; Bennis, M.; Heuson, E.; Roussel, P.; Dumeignil, F.; Paul, S. Design of a multi-well plate for high-throughput characterization of heterogeneous catalysts by XRD, FT-IR, Raman and XRF spectroscopies. *RSC Adv.* **2018**, *8* (71), 40912-40920.
- (46) Löbel, R.; Thienhaus, S.; Savan, A.; Ludwig, A. Combinatorial fabrication and high-throughput characterization of a Ti–Ni–Cu shape memory thin film composition spread. *Mater. Sci. Eng. A* **2008**, *481-482*, 151-155.
- (47) Naujoks, D.; Eggeler, Y. M.; Hallensleben, P.; Frenzel, J.; Fries, S. G.; Palumbo, M.; Koßmann, J.; Hammerschmidt, T.; Pfetzinger-Micklich, J.; Eggeler, G.; et al. Identification of a ternary  $\mu$ -phase in the Co-Ti-W system – An advanced correlative thin-film and bulk combinatorial materials investigation. *Acta Mater.* **2017**, *138*, 100-110.
- (48) Otani, M.; Itaka, K.; Wong-Ng, W.; Schenck, P. K.; Koinuma, H. Development of a high-throughput thermoelectric screening tool for combinatorial thin film libraries. *Appl. Surf. Sci.* **2007**, *254* (3), 765-767.
- (49) Yan, Y. G.; Martin, J.; Wong-Ng, W.; Green, M.; Tang, X. F. A temperature dependent screening tool for high throughput thermoelectric characterization of combinatorial films. *Rev. Sci. Instrum.* **2013**, *84* (11), 115110.
- (50) Otani, M.; Lowhorn, N. D.; Schenck, P. K.; Wong-Ng, W.; Green, M. L.; Itaka, K.; Koinuma, H. A high-throughput thermoelectric power-factor screening tool for rapid construction of thermoelectric property diagrams. *Appl. Phys. Lett.* **2007**, *91* (13), 132102.

- (51) Hintsala, E. D.; Hangen, U.; Stauffer, D. D. High-Throughput Nanoindentation for Statistical and Spatial Property Determination. *JOM* **2018**, 70 (4), 494-503.
- (52) Ling, J.; Chen, W.; Sheng, Y.; Li, W.; Zhang, L.; Du, Y. A MGI-oriented investigation of the Young's modulus and its application to the development of a novel Ti–Nb–Zr–Cr bio-alloy. *Mater. Sci. Eng. C* **2020**, 106, 110265.
- (53) Gong, X.; Yabansu, Y. C.; Collins, P. C.; Kalidindi, S. R. Evaluation of Ti–Mn Alloys for Additive Manufacturing Using High-Throughput Experimental Assays and Gaussian Process Regression. In *Materials*, 2020; Vol. 13.
- (54) Ye, H.; Lee, J.; Jang, J. S.; Bard, A. J. Rapid Screening of BiVO<sub>4</sub>-Based Photocatalysts by Scanning Electrochemical Microscopy (SECM) and Studies of Their Photoelectrochemical Properties. *J. Phys. Chem. C* **2010**, 114 (31), 13322-13328.
- (55) Kimmich, D.; Taffa, D. H.; Dosche, C.; Wark, M.; Wittstock, G. Combinatorial screening of photoanode materials - Uniform platform for compositional arrays and macroscopic electrodes. *Electrochim. Acta* **2018**, 259, 204-212.
- (56) Bash, D.; Cai, Y.; Chellappan, V.; Wong, S. L.; Yang, X.; Kumar, P.; Tan, J. D.; Abutaha, A.; Cheng, J. J. W.; Lim, Y.-F.; et al. Multi-Fidelity High-Throughput Optimization of Electrical Conductivity in P3HT-CNT Composites. *Adv. Funct. Mater.* **2021**, 31 (36), 2102606.
- (57) Harillo-Baños, A.; Fan, Q.; Riera-Galindo, S.; Wang, E.; Inganäs, O.; Campoy-Quiles, M. High-Throughput Screening of Blade-Coated Polymer:Polymer Solar Cells: Solvent Determines Achievable Performance. *ChemSusChem* **2022**, 15 (4), e202101888.
- (58) Shi, Y.; Villani, E.; Chen, Y.; Zhou, Y.; Chen, Z.; Hussain, A.; Xu, G.; Inagi, S. High-Throughput Electrosynthesis of Gradient Polypyrrole Film Using a Single-Electrode Electrochemical System. *Anal. Chem.* **2023**, 95 (2), 1532-1540.

- (59) Wang, R.; Lüer, L.; Langner, S.; Heumueller, T.; Forberich, K.; Zhang, H.; Hauch, J.; Li, N.; Brabec, C. J. Understanding the Microstructure Formation of Polymer Films by Spontaneous Solution Spreading Coating with a High-Throughput Engineering Platform. *ChemSusChem* **2021**, *14* (17), 3590-3598.
- (60) Baudis, S.; Behl, M. High-Throughput and Combinatorial Approaches for the Development of Multifunctional Polymers. *Macromol. Rapid Commun.* **2022**, *43* (12), 2100400.
- (61) Zhang, R.; Liberski, A.; Sanchez-Martin, R.; Bradley, M. Microarrays of Over 2000 Hydrogels – Identification of Substrates for Cellular Trapping and Thermally Triggered Release. *Biomaterials* **2009**, *30* (31), 6193-6201.
- (62) Rosenfeld, A.; Oelschlaeger, C.; Thelen, R.; Heissler, S.; Levkin, P. A. Miniaturized High-Throughput Synthesis and Screening of Responsive Hydrogels Using Nanoliter Compartments. *Mater. Today Bio* **2020**, *6*, 100053.
- (63) Yang, J.; Ran, Y.; Huang, L.; Ren, C.; Hao, X.; Ma, L.; Zhang, D. High-throughput screening of zwitterion-based coatings towards improved mechanical stability and drug-loading capacity. *npj Mater. Degrad.* **2023**, *7* (1), 51.
- (64) Ranga, A.; Gobaa, S.; Okawa, Y.; Mosiewicz, K.; Negro, A.; Lutolf, M. P. 3D Niche Microarrays for Systems-Level Analyses of Cell Fate. *Nat. Commun.* **2014**, *5* (1), 4324.
- (65) Xu, F.; Corbett, B.; Bell, S.; Zhang, C. Y.; Hartono, M. B.; Farsangi, Z. J.; MacGregor, J.; Hoare, T. High-Throughput Synthesis, Analysis, and Optimization of Injectable Hydrogels for Protein Delivery. *Biomacromolecules* **2020**, *21* (1), 214-229.
- (66) Kim, T. H.; An, D. B.; Oh, S. H.; Kang, M. K.; Song, H. H.; Lee, J. H. Creating stiffness gradient polyvinyl alcohol hydrogel using a simple gradual freezing–thawing method to investigate stem cell differentiation behaviors. *Biomaterials* **2015**, *40*, 51-60.

- (67) Yi, Z.; Zhang, Y.; Kootala, S.; Hilborn, J.; Ossipov, D. A. Hydrogel Patterning by Diffusion through the Matrix and Subsequent Light-Triggered Chemical Immobilization. *ACS Appl. Mater. Interfaces* **2015**, 7 (2), 1194-1206.
- (68) Du, Y.; Hancock, M. J.; He, J.; Villa-Urbe, J. L.; Wang, B.; Cropek, D. M.; Khademhosseini, A. Convection-driven generation of long-range material gradients. *Biomaterials* **2010**, 31 (9), 2686-2694.
- (69) Domachuk, P.; Tsioris, K.; Omenetto, F. G.; Kaplan, D. L. Bio-microfluidics: Biomaterials and Biomimetic Designs. *Adv. Mater.* **2010**, 22 (2), 249-260.
- (70) Barata, D.; van Blitterswijk, C.; Habibovic, P. High-Throughput Screening Approaches and Combinatorial Development of Biomaterials Using Microfluidics. *Acta Biomater.* **2016**, 34, 1-20.
- (71) Kumachev, A.; Greener, J.; Tumarkin, E.; Eiser, E.; Zandstra, P. W.; Kumacheva, E. High-Throughput Generation of Hydrogel Microbeads With Varying Elasticity for Cell Encapsulation. *Biomaterials* **2011**, 32 (6), 1477-1483.
- (72) Zhang, R.; Liberski, A.; Khan, F.; Diaz-Mochon, J. J.; Bradley, M. Inkjet Fabrication of Hydrogel Microarrays Using in situ Nanolitre-Scale Polymerisation. *Chem. Commun.* **2008**, (11), 1317-1319.
- (73) Hao, H.; Huang, J.; Liu, P.; Xue, Y.; Wang, J.; Ren, K.; Jin, Q.; Ji, J.; Greiner, A.; Agarwal, S. Rapid build-up of high-throughput screening microarrays with biochemistry gradients via light-induced thiol–ene “click” chemistry. *J. Mater. Chem. B* **2021**, 9 (13), 3032-3037.
- (74) Guimarães, C. F.; Gasperini, L.; Ribeiro, R. S.; Carvalho, A. F.; Marques, A. P.; Reis, R. L. High-Throughput Fabrication of Cell-Laden 3D Biomaterial Gradients. *Mater. Horiz.* **2020**, 7 (9), 2414-2421.

- (75) Liu, A. L.; Dogan-Guner, E. M.; McBride, M.; Venkatesh, R.; Gonzalez, M. A.; Reichmanis, E.; Grover, M.; Meredith, J. C. Composition Gradient High-Throughput Polymer Libraries Enabled by Passive Mixing and Elevated Temperature Operability. *Chem. Mater.* **2022**, *34* (15), 6659-6670.
- (76) Burdick, J. A.; Khademhosseini, A.; Langer, R. Fabrication of Gradient Hydrogels Using a Microfluidics/Photopolymerization Process. *Langmuir* **2004**, *20* (13), 5153-5156.
- (77) Ding, Y.; Tang, H. Q.; Zhang, C. H.; Li, W. X.; Li, G.; Zhang, Y.; Xu, C.; Zhao, F.; Guo, Q. Y.; Guo, C. F.; et al. High-Throughput Screening of Self-Healable Polysulfobetaine Hydrogels and their Applications in Flexible Electronics. *Adv. Funct. Mater.* **2021**, *31* (18), 11.
- (78) Pollice, R.; dos Passos Gomes, G.; Aldeghi, M.; Hickman, R. J.; Krenn, M.; Lavigne, C.; Lindner-D'Addario, M.; Nigam, A.; Ser, C. T.; Yao, Z.; et al. Data-Driven Strategies for Accelerated Materials Design. *Acc. Chem. Res.* **2021**, *54* (4), 849-860.
- (79) Rooney, M. B.; MacLeod, B. P.; Oldford, R.; Thompson, Z. J.; White, K. L.; Tungjunyatham, J.; Stankiewicz, B. J.; Berlinguette, C. P. A self-driving laboratory designed to accelerate the discovery of adhesive materials. *Digit. Discov.* **2022**, *1* (4), 382-389.
- (80) Lui, Y. S.; Sow, W. T.; Tan, L. P.; Wu, Y.; Lai, Y.; Li, H. 4D printing and stimuli-responsive materials in biomedical aspects. *Acta Biomater.* **2019**, *92*, 19-36.
- (81) Kuang, X.; Roach, D. J.; Wu, J.; Hamel, C. M.; Ding, Z.; Wang, T.; Dunn, M. L.; Qi, H. J. Advances in 4D Printing: Materials and Applications. *Adv. Funct. Mater.* **2019**, *29* (2).
- (82) Zhang, L.; Yang, G.; Johnson, B. N.; Jia, X. Three-dimensional (3D) printed scaffold and material selection for bone repair. *Acta Biomater.* **2019**, *84*, 16-33.

- (83) Döring, A.; Birnbaum, W.; Kuckling, D. Responsive hydrogels – structurally and dimensionally optimized smart frameworks for applications in catalysis, micro-system technology and material science. *Chem. Soc. Rev.* **2013**, 42 (17), 7391-7420.
- (84) Hoffman, A. S. Stimuli-responsive polymers: Biomedical applications and challenges for clinical translation. *Adv. Drug Deliv. Rev.* **2013**, 65 (1), 10-16.
- (85) Dong, Y.; Wang, S.; Ke, Y.; Ding, L.; Zeng, X.; Magdassi, S.; Long, Y. 4D Printed Hydrogels: Fabrication, Materials, and Applications. *Adv. Mater. Technol.* **2020**, 5 (6).
- (86) Champeau, M.; Heinze, D. A.; Viana, T. N.; de Souza, E. R.; Chinellato, A. C.; Titotto, S. 4D Printing of Hydrogels: A Review. *Adv. Funct. Mater.* **2020**, 30 (31).
- (87) Zhou, W.; Qiao, Z.; Nazarzadeh Zare, E.; Huang, J.; Zheng, X.; Sun, X.; Shao, M.; Wang, H.; Wang, X.; Chen, D.; et al. 4D-Printed Dynamic Materials in Biomedical Applications: Chemistry, Challenges, and Their Future Perspectives in the Clinical Sector. *J. Med. Chem.* **2020**, 63 (15), 8003-8024.
- (88) Halperin, A.; Kröger, M.; Winnik, F. M. Poly(N-isopropylacrylamide) Phase Diagrams: Fifty Years of Research. *Angew. Chem. Int. Ed.* **2015**, 54 (51), 15342-15367.
- (89) Bakarich, S. E.; Gorkin III, R.; Panhuis, M. i. h.; Spinks, G. M. 4D Printing with Mechanically Robust, Thermally Actuating Hydrogels. *Macromol. Rapid Commun.* **2015**, 36 (12), 1211-1217.
- (90) Naficy, S.; Gately, R.; Gorkin III, R.; Xin, H.; Spinks, G. M. 4D Printing of Reversible Shape Morphing Hydrogel Structures. *Macromol. Mater. Eng.* **2017**, 302 (1).
- (91) Deng, Z.; Guo, Y.; Zhao, X.; Ma, P. X.; Guo, B. Multifunctional Stimuli-Responsive Hydrogels with Self-Healing, High Conductivity, and Rapid Recovery through Host-Guest Interactions. *Chem. Mater.* **2018**, 30 (5), 1729-1742.

- (92) Han, D.; Lu, Z.; Chester, S. A.; Lee, H. Micro 3D Printing of a Temperature-Responsive Hydrogel Using Projection Micro-Stereolithography. *Sci. Rep.* **2018**, 8 (1).
- (93) Sydney Gladman, A.; Matsumoto, E. A.; Nuzzo, R. G.; Mahadevan, L.; Lewis, J. A. Biomimetic 4D printing. *Nat. Mater.* **2016**, 15 (4), 413-418.
- (94) Liu, J.; Erol, O.; Pantula, A.; Liu, W.; Jiang, Z.; Kobayashi, K.; Chatterjee, D.; Hibino, N.; Romer, L. H.; Kang, S. H.; et al. Dual-Gel 4D Printing of Bioinspired Tubes. *ACS Appl. Mater. Interfaces* **2019**, 11 (8), 8492-8498.
- (95) Liu, S.; Li, L. Ultrastretchable and Self-Healing Double-Network Hydrogel for 3D Printing and Strain Sensor. *ACS Appl. Mater. Interfaces* **2017**, 9 (31), 26429-26437.
- (96) Zhou, Y.; Wan, C.; Yang, Y.; Yang, H.; Wang, S.; Dai, Z.; Ji, K.; Jiang, H.; Chen, X.; Long, Y. Highly Stretchable, Elastic, and Ionic Conductive Hydrogel for Artificial Soft Electronics. *Adv. Funct. Mater.* **2019**, 29 (1), 1806220.
- (97) Hong, S.; Sycks, D.; Chan, H. F.; Lin, S.; Lopez, G. P.; Guilak, F.; Leong, K. W.; Zhao, X. 3D Printing of Highly Stretchable and Tough Hydrogels into Complex, Cellularized Structures. *Adv. Mater.* **2015**, 27 (27), 4035-4040.
- (98) Rasool, A.; Ata, S.; Islam, A. Stimuli responsive biopolymer (chitosan) based blend hydrogels for wound healing application. *Carbohydr. Polym.* **2019**, 203, 423-429.
- (99) Swelling Behaviors of 3D Printed Hydrogel and Hydrogel-Microcarrier Composite Scaffolds. *Tissue Eng. Part A* **2021**, 27 (11-12), 665-678.
- (100) Jimenez-Vergara, A. C.; Lewis, J.; Hahn, M. S.; Munoz-Pinto, D. J. An improved correlation to predict molecular weight between crosslinks based on equilibrium degree of swelling of hydrogel networks. *J. Biomed. Mater. Res. B Appl. Biomater.* **2018**, 106 (3), 1339-1348.

- (101) Chang, C. Y.; Duan, B.; Cai, J.; Zhang, L. N. Superabsorbent hydrogels based on cellulose for smart swelling and controllable delivery. *Eur. Polym. J.* **2010**, *46* (1), 92-100.
- (102) Shi, P.; Laude, A.; Yeong, W. Y. Investigation of cell viability and morphology in 3D bio-printed alginate constructs with tunable stiffness. *J. Biomed. Mater. Res. A* **2017**, *105* (4), 1009-1018.
- (103) Hahn, L.; Maier, M.; Stahlhut, P.; Beudert, M.; Flegler, V.; Forster, S.; Altmann, A.; Töppke, F.; Fischer, K.; Seiffert, S.; et al. Inverse Thermogelation of Aqueous Triblock Copolymer Solutions into Macroporous Shear-Thinning 3D Printable Inks. *ACS Appl. Mater. Interfaces* **2020**, *12* (11), 12445-12456.
- (104) Nishi, K.; Hiroi, T.; Hashimoto, K.; Fujii, K.; Han, Y.-S.; Kim, T.-H.; Katsumoto, Y.; Shibayama, M. SANS and DLS Study of Tacticity Effects on Hydrophobicity and Phase Separation of Poly(N-isopropylacrylamide). *Macromolecules* **2013**, *46* (15), 6225-6232.
- (105) Saunders, B. R. On the Structure of Poly(N-isopropylacrylamide) Microgel Particles. *Langmuir* **2004**, *20* (10), 3925-3932.
- (106) Shibayama, M. Small-angle neutron scattering on polymer gels: phase behavior, inhomogeneities and deformation mechanisms. *Polym. J.* **2011**, *43* (1), 18-34.
- (107) Pujals, S.; Feiner-Gracia, N.; Delcanale, P.; Voets, I.; Albertazzi, L. Super-resolution microscopy as a powerful tool to study complex synthetic materials. *Nat. Rev. Chem.* **2019**, *3* (2), 68-84.
- (108) Kubota, R.; Torigoe, S.; Liu, S.; Hamachi, I. In Situ Real-time Confocal Imaging of a Self-assembling Peptide-grafted Polymer Showing pH-responsive Hydrogelation. *Chem. Lett.* **2020**, *49* (11), 1319-1323.

- (109) Si, T. B.; Wang, Y. X.; Wei, W.; Lv, P.; Ma, G. H.; Su, Z. G. Effect of acrylic acid weight percentage on the pore size in poly(N-Isopropyl acrylamide-co-acrylic acid) microspheres. *React. Funct. Polym.* **2011**, *71* (7), 728-735.
- (110) Mulakkal, M. C.; Trask, R. S.; Ting, V. P.; Seddon, A. M. Responsive cellulose-hydrogel composite ink for 4D printing. *Mater. Des.* **2018**, *160*, 108-118.
- (111) Shin, S.; Hyun, J. Rheological properties of cellulose nanofiber hydrogel for high-fidelity 3D printing. *Carbohydr. Polym.* **2021**, *263*, 117976.
- (112) Zhu, F.; Cheng, L.; Yin, J.; Wu, Z. L.; Qian, J.; Fu, J.; Zheng, Q. 3D Printing of Ultratough Polyion Complex Hydrogels. *ACS Appl. Mater. Interfaces* **2016**, *8* (45), 31304-31310.
- (113) Karvinen, J.; Ihalainen, T. O.; Calejo, M. T.; Jönkkäri, I.; Kellomäki, M. Characterization of the microstructure of hydrazone crosslinked polysaccharide-based hydrogels through rheological and diffusion studies. *Mater. Sci. Eng. C* **2019**, *94*, 1056-1066.
- (114) Haring, A. P.; Singh, M.; Koh, M.; Cesewski, E.; Dillard, D. A.; James Kong, Z.; Johnson, B. N. Real-time characterization of hydrogel viscoelastic properties and sol-gel phase transitions using cantilever sensors. *J. Rheol.* **2020**, *64* (4), 837-850.
- (115) Cesewski, E.; Singh, M.; Liu, Y.; Zhang, J.; Haring, A. P.; Johnson, B. N. Real-time monitoring of hydrogel rheological property changes and gelation processes using high-order modes of cantilever sensors. *J. Appl. Phys.* **2020**, *128* (17).
- (116) Singh, M.; Zhang, J.; Bethel, K.; Liu, Y.; Davis, E. M.; Zeng, H.; Kong, Z.; Johnson, B. N. Closed-Loop Controlled Photopolymerization of Hydrogels. *ACS Appl. Mater. Interfaces* **2021**, *13* (34), 40365-40378.
- (117) Chirra, H. D.; Hilt, J. Z. Nanoscale Characterization of the Equilibrium and Kinetic Response of Hydrogel Structures. *Langmuir* **2010**, *26* (13), 11249-11257.

- (118) Matsui, S.; Nishizawa, Y.; Uchihashi, T.; Suzuki, D. Monitoring Thermoresponsive Morphological Changes in Individual Hydrogel Microspheres. *ACS Omega* **2018**, 3 (9), 10836-10842.
- (119) Sharma, H.; Lakshmanan, R. S.; Johnson, B. N.; Mutharasan, R. Piezoelectric cantilever sensors with asymmetric anchor exhibit picogram sensitivity in liquids. *Sens. Actuators B: Chem.* **2011**, 153 (1), 64-70.
- (120) Mather, M. L.; Rides, M.; Allen, C. R. G.; Tomlins, P. E. Liquid viscoelasticity probed by a mesoscale piezoelectric bimorph cantilever. *J. Rheol.* **2012**, 56 (1), 99-112.
- (121) Chippada, U.; Yurke, B.; Langrana, N. A. Simultaneous determination of Young's modulus, shear modulus, and Poisson's ratio of soft hydrogels. *J. Mater. Res.* **2010**, 25 (3), 545-555.
- (122) Gotou, R.; Shimizu, K. Slip Fracture and Shear Strength of Bentonite Gels. *Bulletin of the Institute for Chemical Research, Kyoto University* **1966**, 43 (4-5), 380-387.
- (123) Kuo, C. K.; Ma, P. X. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: Part 1. Structure, gelation rate and mechanical properties. *Biomaterials* **2001**, 22 (6), 511-521.
- (124) Chimene, D.; Lennox, K. K.; Kaunas, R. R.; Gaharwar, A. K. Advanced Bioinks for 3D Printing: A Materials Science Perspective. *Ann. Biomed. Eng.* **2016**, 44 (6), 2090-2102.
- (125) Bonino, C. A.; Samorezov, J. E.; Jeon, O.; Alsberg, E.; Khan, S. A. Real-time in situ rheology of alginate hydrogel photocrosslinking. *Soft Matter* **2011**, 7 (24), 11510-11517.
- (126) Murata, H. Rheology - Theory and Application to Biomaterials. In *Polymerization*, Gomes, A. d. S. Ed.; IntechOpen, 2012.

- (127) Xing, Z. Y.; Caciagli, A.; Cao, T. Y.; Stoev, I.; Zupkauskas, M.; O'Neill, T.; Wenzel, T.; Lamboll, R.; Liu, D. S.; Eiser, E. Microrheology of DNA hydrogels. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (32), 8137-8142.
- (128) Winter, H. H.; Chambon, F. Analysis of Linear Viscoelasticity of a Crosslinking Polymer at the Gel Point. *J. Rheol.* **1986**, *30* (2), 367-382.
- (129) Janmey, P. A.; Georges, P. C.; Hvidt, S. Basic Rheology for Biologists. In *Methods in Cell Biology*, Vol. 83; Academic Press, 2007; pp 1-27.
- (130) Delgado, D. E.; Sturdy, L. F.; Burkhart, C. W.; Shull, K. R. Validation of quartz crystal rheometry in the megahertz frequency regime. *J. Polym. Sci. B: Polym. Phys.* **2019**, *57* (18), 1246-1254.
- (131) Safakas, K.; Saravanou, S.-F.; Iatridi, Z.; Tsitsilianis, C. Alginate-g-PNIPAM-Based Thermo/Shear-Responsive Injectable Hydrogels: Tailoring the Rheological Properties by Adjusting the LCST of the Grafting Chains. *Int. J. Mol. Sci.* **2021**, *22* (8), 3824.
- (132) Soledad Lencina, M. M.; Iatridi, Z.; Villar, M. A.; Tsitsilianis, C. Thermoresponsive hydrogels from alginate-based graft copolymers. *Eur. Polym. J.* **2014**, *61*, 33-44.
- (133) Jian, Y.; Wu, B.; Yang, X.; Peng, Y.; Zhang, D.; Yang, Y.; Qiu, H.; Lu, H.; Zhang, J.; Chen, T. Stimuli-responsive hydrogel sponge for ultrafast responsive actuator. *Supramol. Mater.* **2022**, *1*, 100002.
- (134) Mennen, S. M.; Alhambra, C.; Allen, C. L.; Barberis, M.; Berritt, S.; Brandt, T. A.; Campbell, A. D.; Castanon, J.; Cherney, A. H.; Christensen, M.; et al. The Evolution of High-Throughput Experimentation in Pharmaceutical Development and Perspectives on the Future. *Org. Process Res. Dev.* **2019**, *23* (6), 1213-1242.

- (135) Yang, Y.-H.; Khan, Z.; Ma, C.; Lim, H. J.; Smith Callahan, L. A. Optimization of Adhesive Conditions for Neural Differentiation of Murine Embryonic Stem Cells Using Hydrogels Functionalized With Continuous Ile-Lys-Val-Ala-Val Concentration Gradients. *Acta Biomater.* **2015**, *21*, 55-62.
- (136) Zhang, J.; Liu, Y.; Chandra Sekhar.P, D.; Singh, M.; Tong, Y.; Kucukdeger, E.; Yoon, H. Y.; Haring, A. P.; Roman, M.; Kong, Z.; et al. Rapid, Autonomous High-Throughput Characterization of Hydrogel Rheological Properties via Automated Sensing and Physics-Guided Machine Learning. *Appl. Mater. Today* **2023**, *30*, 101720.
- (137) de Rutte, J. M.; Koh, J.; Di Carlo, D. Scalable High-Throughput Production of Modular Microgels for In Situ Assembly of Microporous Tissue Scaffolds. *Adv. Funct. Mater.* **2019**, *29* (25), 10.
- (138) Wang, M.; Nai, M. H.; Huang, R. Y.-J.; Leo, H. L.; Lim, C. T.; Chen, C.-H. High-Throughput Functional Profiling of Single Adherent Cells via Hydrogel Drop-Screen. *Lab Chip* **2021**, *21* (4), 764-774.
- (139) André, E.; Pannacci, N.; Dalmazzone, C.; Colin, A. A New Way to Measure Viscosity in Droplet-Based Microfluidics for High Throughput Analysis. *Soft Matter* **2019**, *15* (3), 504-514.
- (140) Oevreeide, I. H.; Szydlak, R.; Luty, M.; Ahmed, H.; Prot, V.; Skallerud, B. H.; Zemla, J.; Lekka, M.; Stokke, B. T. On the Determination of Mechanical Properties of Aqueous Microgels-Towards High-Throughput Characterization. *Gels* **2021**, *7* (2), 18.
- (141) Marquez, J. P.; Legant, W.; Lam, V.; Cayemberg, A.; Elson, E.; Wakatsuki, T. High-Throughput Measurements of Hydrogel Tissue Construct Mechanics. *Tissue Eng. Part C-Methods* **2009**, *15* (2), 181-190.

- (142) Haring, A. P.; Singh, M.; Koh, M.; Cesewski, E.; Dillard, D. A.; Kong, Z. J.; Johnson, B. N. Real-Time Characterization of Hydrogel Viscoelastic Properties and Sol-Gel Phase Transitions Using Cantilever Sensors. *J. Rheol.* **2020**, *64* (4), 837-850.
- (143) Cesewski, E.; Singh, M.; Liu, Y.; Zhang, J.; Haring, A. P.; Johnson, B. N. Real-Time Monitoring of Hydrogel Rheological Property Changes and Gelation Processes Using High-Order Modes of Cantilever Sensors. *J. Appl. Phys.* **2020**, *128* (17), 174502.
- (144) Liu, Y.; Bethel, K.; Singh, M.; Zhang, J.; Ashkar, R.; Davis, E. M.; Johnson, B. N. Comparison of Bulk- vs Layer-by-Layer-Cured Stimuli-Responsive PNIPAM–Alginate Hydrogel Dynamic Viscoelastic Property Response via Embedded Sensors. *ACS Appl. Polym. Mater.* **2022**, *4* (8), 5596-5607.
- (145) Johnson, B. N.; Mutharasan, R. Persistence of Bending and Torsional Modes in Piezoelectric-Excited Millimeter-Sized Cantilever (PEMC) Sensors in Viscous Liquids - 1 to 103 cP. *J. Appl. Phys.* **2011**, *109* (6), 066105.
- (146) Haring, A. P.; Khan, A. U.; Liu, G.; Johnson, B. N. 3D Printed Functionally Graded Plasmonic Constructs. *Adv. Opt. Mater.* **2017**, *5* (18), 1700367.
- (147) Killion, J. A.; Geever, L. M.; Devine, D. M.; Kennedy, J. E.; Higginbotham, C. L. Mechanical Properties and Thermal Behaviour of PEGDMA Hydrogels for Potential Bone Regeneration Application. *J. Mech. Behav. Biomed. Mater.* **2011**, *4* (7), 1219-1227.
- (148) Burke, G.; Barron, V.; Geever, T.; Geever, L.; Devine, D. M.; Higginbotham, C. L. Evaluation of the Materials Properties, Stability and Cell Response of a Range of PEGDMA Hydrogels for Tissue Engineering Applications. *J. Mech. Behav. Biomed. Mater.* **2019**, *99*, 1-10.
- (149) de Lima, G. G.; Elter, J. K.; Chee, B. S.; Magalhaes, W. L. E.; Devine, D. M.; Nugent, M. J. D.; de Sa, M. J. C. A Tough and Novel Dual-Response PAA/P(NiPAAM-co-PEGDMA) IPN

Hydrogels With Ceramics by Photopolymerization for Consolidation of Bone Fragments Following Fracture. *Biomed. Mater.* **2019**, *14* (5), 054101.

(150) Mather, M. L.; Rides, M.; Allen, C. R. G.; Tomlins, P. E. Liquid Viscoelasticity Probed by a Mesoscale Piezoelectric Bimorph Cantilever. *J. Rheol.* **2011**, *56* (1), 99-112.

(151) Yang, J.; Han, C.-R.; Duan, J.-F.; Ma, M.-G.; Zhang, X.-M.; Xu, F.; Sun, R.-C.; Xie, X.-M. Studies on the Properties and Formation Mechanism of Flexible Nanocomposite Hydrogels From Cellulose Nanocrystals and Poly(acrylic acid). *J. Mater. Chem.* **2012**, *22* (42), 22467-22480.

(152) Yang, J.; Han, C.-R. Dynamics of Silica-Nanoparticle-Filled Hybrid Hydrogels: Nonlinear Viscoelastic Behavior and Chain Entanglement Network. *J. Phys. Chem. C* **2013**, *117* (39), 20236-20243.

(153) Steffens, J.; Fazio, M. A.; Cavalcoli, D.; Terheiden, B. Multi-Characterization Study of Interface Passivation Quality of Amorphous Sub-Stoichiometric Silicon Oxide and Silicon Oxynitride Layers for Photovoltaic Applications. *Sol. Energy Mater. Sol. Cells* **2018**, *187*, 104-112.

(154) Wu, J. X.; Yang, S.; Cai, W.; Bi, Z. F.; Shang, G. Y.; Yao, J. N. Multi-Characterization of LiCoO<sub>2</sub> Cathode Films Using Advanced AFM-Based Techniques With High Resolution. *Sci. Rep.* **2017**, *7*, 11164.

(155) Abbas, A.; Vivien, C.; Bocquet, B.; Guillochon, D.; Supiot, P. Preparation and Multi-Characterization of Plasma Polymerized Allylamine Films. *Plasma Process. Polym.* **2009**, *6* (9), 593-604.

(156) Chen, Q.; Chen, H.; Zhu, L.; Zheng, J. Fundamentals of Double Network Hydrogels. *J. Mater. Chem. B* **2015**, *3* (18), 3654-3676.

- (157) Tang, S.; Yang, J.; Lin, L.; Peng, K.; Chen, Y.; Jin, S.; Yao, W. Construction of Physically Crosslinked Chitosan/Sodium Alginate/Calcium Ion Double-Network Hydrogel and Its Application to Heavy Metal Ions Removal. *Chem. Eng. J.* **2020**, *393*, 124728.
- (158) Wiener, C. G.; Wang, C.; Liu, Y.; Weiss, R. A.; Vogt, B. D. Nanostructure Evolution During Relaxation From a Large Step Strain in a Supramolecular Copolymer-Based Hydrogel: A SANS Investigation. *Macromolecules* **2017**, *50* (4), 1672-1680.
- (159) Yu, L.; Yakubov, G. E.; Gilbert, E. P.; Sewell, K.; van de Meene, A. M. L.; Stokes, J. R. Multi-Scale Assembly of Hydrogels Formed by Highly Branched Arabinoxylans From *Plantago Ovata* Seed Mucilage Studied by USANS/SANS and Rheology. *Carbohydr. Polym.* **2019**, *207*, 333-342.
- (160) Nishi, K.; Shibayama, M. Probe-SAXS on Hydrogels Under Elongation. *Soft Matter* **2016**, *12* (24), 5334-5339.
- (161) Huang, L.; Ding, L.; Zhou, J.; Chen, S.; Chen, F.; Zhao, C.; Xu, J.; Hu, W.; Ji, J.; Xu, H.; et al. One-step Rapid Quantification of SARS-CoV-2 Virus Particles via Low-Cost Nanoplasmonic Sensors in Generic Microplate Reader and Point-of-Care Device. *Biosens. Bioelectron.* **2021**, *171*, 112685.
- (162) Mauri, M.; Vecchione, S.; Fritz, G. Deconvolution of Luminescence Cross-Talk in High-Throughput Gene Expression Profiling. *ACS Synth. Biol.* **2019**, *8* (6), 1361-1370.
- (163) Yeow, J.; Joshi, S.; Chapman, R.; Boyer, C. A Self-Reporting Photocatalyst for Online Fluorescence Monitoring of High Throughput RAFT Polymerization. *Angew. Chem. Int. Ed.* **2018**, *57* (32), 10102-10106.

- (164) Nakamura, T.; Mizukami, S.; Tanaka, M.; Kikuchi, K. Efficient Formation of Luminescent Lanthanide(III) Complexes by Solid-Phase Synthesis and On-Resin Screening. *Chem.-Asian J.* **2013**, *8* (11), 2685-2690.
- (165) Rahaman, M.; Aldalbahi, A.; Govindasami, P.; Khanam, N. P.; Bhandari, S.; Feng, P.; Altalhi, T. A New Insight in Determining the Percolation Threshold of Electrical Conductivity for Extrinsicly Conducting Polymer Composites Through Different Sigmoidal Models. *Polymers* **2017**, *9* (10), 527.
- (166) Moud, A. A.; Kamkar, M.; Sanati-Nezhad, A.; Hejazi, S. H.; Sundararaj, U. Viscoelastic Properties of Poly (vinyl alcohol) Hydrogels With Cellulose Nanocrystals Fabricated Through Sodium Chloride Addition: Rheological Evidence of Double Network Formation. *Colloids Surf. A Physicochem. Eng. Asp.* **2021**, *609*, 125577.
- (167) Duan, Y.; Zhao, Y.; Ai, S.; Qiu, D.; Wang, X.; Qu, X.; Yang, Z. Programmable Processing Toward Stiff Composite Hydrogels. *Macromolecules* **2022**, *55* (16), 7071-7079.
- (168) Kayser, L. V.; Lipomi, D. J. Stretchable Conductive Polymers and Composites Based on PEDOT and PEDOT:PSS. *Adv. Mater.* **2019**, *31* (10), 1806133.
- (169) Sun, K.; Zhang, S.; Li, P.; Xia, Y.; Zhang, X.; Du, D.; Isikgor, F. H.; Ouyang, J. Review on Application of PEDOTs and PEDOT:PSS in Energy Conversion and Storage Devices. *J. Mater. Sci. Mater. Electron.* **2015**, *26* (7), 4438-4462.
- (170) Fan, X.; Nie, W.; Tsai, H.; Wang, N.; Huang, H.; Cheng, Y.; Wen, R.; Ma, L.; Yan, F.; Xia, Y. PEDOT:PSS for Flexible and Stretchable Electronics: Modifications, Strategies, and Applications. *Adv. Sci.* **2019**, *6* (19), 1900813.
- (171) Kaur, G.; Adhikari, R.; Cass, P.; Bown, M.; Gunatillake, P. Electrically Conductive Polymers and Composites for Biomedical Applications. *RSC Adv.* **2015**, *5* (47), 37553-37567.

- (172) Heo, D. N.; Lee, S.-J.; Timsina, R.; Qiu, X.; Castro, N. J.; Zhang, L. G. Development of 3D Printable Conductive Hydrogel With Crystallized PEDOT:PSS for Neural Tissue Engineering. *Mater. Sci. Eng. C* **2019**, *99*, 582-590.
- (173) Guex, A. G.; Puetzer, J. L.; Armgarth, A.; Littmann, E.; Stavriniidou, E.; Giannelis, E. P.; Malliaras, G. G.; Stevens, M. M. Highly Porous Scaffolds of PEDOT:PSS for Bone Tissue Engineering. *Acta Biomater.* **2017**, *62*, 91-101.
- (174) Khalili, A.; Noziere, R.; Tuberty-Vaughan, E.; Freeman, J. W. Self-Actuating Multilayer Scaffold for Skeletal Muscle Tissue Engineering. *Polym. Adv. Technol.* **2022**, *33* (10), 3228-3237.
- (175) Abdullah, H.; Phairatana, T. A Study on the Performances of PEGDA and PEDOT: PSS Composite Resin for 3D-Printed Biosensor Applications. *ECS Trans.* **2022**, *107* (1), 15001.
- (176) Lopez-Larrea, N.; Criado-Gonzalez, M.; Dominguez-Alfaro, A.; Alegret, N.; Agua, I. d.; Marchiori, B.; Mecerreyes, D. Digital Light 3D Printing of PEDOT-Based Photopolymerizable Inks for Biosensing. *ACS Appl. Polym. Mater.* **2022**, *4* (9), 6749-6759.
- (177) Polino, G.; Lubrano, C.; Scognamiglio, P.; Mollo, V.; De Martino, S.; Ciccone, G.; Matino, L.; Langella, A.; Netti, P.; Di Carlo, A.; et al. Synthesis and Characterization of PEDOT-PEGDA Blends for Bioelectronic Applications: Surface Properties and Effects on Cell Morphology. *Flex. Print. Electron.* **2020**, *5* (1), 014012.
- (178) de Pablo, J. J.; Jackson, N. E.; Webb, M. A.; Chen, L.-Q.; Moore, J. E.; Morgan, D.; Jacobs, R.; Pollock, T.; Schlom, D. G.; Toberer, E. S.; et al. New frontiers for the materials genome initiative. *npj Comput. Mater.* **2019**, *5* (1), 41.
- (179) Maier, W. F.; Stöwe, K.; Sieg, S. Combinatorial and High-Throughput Materials Science. *Angew. Chem. Int. Ed.* **2007**, *46* (32), 6016-6067.

- (180) Ferguson, A. L.; Brown, K. A. Data-Driven Design and Autonomous Experimentation in Soft and Biological Materials Engineering. *Annu. Rev. Chem. Biomol. Eng.* **2022**, *13* (1), 25-44.
- (181) Caskey, C. M.; Richards, R. M.; Ginley, D. S.; Zakutayev, A. Thin film synthesis and properties of copper nitride, a metastable semiconductor. *Mater. Horiz.* **2014**, *1* (4), 424-430.
- (182) Pavan, M.; Rühle, S.; Ginsburg, A.; Keller, D. A.; Barad, H.-N.; Sberna, P. M.; Nunes, D.; Martins, R.; Anderson, A. Y.; Zaban, A.; et al. TiO<sub>2</sub>/Cu<sub>2</sub>O all-oxide heterojunction solar cells produced by spray pyrolysis. *Sol. Energy Mater. Sol. Cells* **2015**, *132*, 549-556.
- (183) Ding, S.; Gregoire, J.; Vlassak, J. J.; Schroers, J. Solidification of Au-Cu-Si alloys investigated by a combinatorial approach. *J. Appl. Phys.* **2012**, *111* (11), 114901.
- (184) Gregoire, J. M.; Xiang, C.; Liu, X.; Marcin, M.; Jin, J. Scanning droplet cell for high throughput electrochemical and photoelectrochemical measurements. *Rev. Sci. Instrum.* **2013**, *84* (2), 024102.
- (185) Adhikari, T.; Hebert, A.; Adamič, M.; Yao, J.; Potts, K.; McCalla, E. Development of High-Throughput Methods for Sodium-Ion Battery Cathodes. *ACS Comb. Sci.* **2020**, *22* (6), 311-318.
- (186) Butler, K. T.; Davies, D. W.; Cartwright, H.; Isayev, O.; Walsh, A. Machine learning for molecular and materials science. *Nature* **2018**, *559* (7715), 547-555.
- (187) Cai, J.; Chu, X.; Xu, K.; Li, H.; Wei, J. Machine learning-driven new material discovery. *Nanoscale Adv.* **2020**, *2* (8), 3115-3130.
- (188) Saal, J. E.; Oliynyk, A. O.; Meredig, B. Machine Learning in Materials Discovery: Confirmed Predictions and Their Underlying Approaches. *Annu. Rev. Mater. Res.* **2020**, *50* (1), 49-69.
- (189) Juan, Y.; Dai, Y.; Yang, Y.; Zhang, J. Accelerating materials discovery using machine learning. *J. Mater. Res. Technol.* **2021**, *79*, 178-190.

- (190) Suh, C.; Fare, C.; Warren, J. A.; Pyzer-Knapp, E. O. Evolving the Materials Genome: How Machine Learning Is Fueling the Next Generation of Materials Discovery. *Annu. Rev. Mater. Res.* **2020**, *50* (1), 1-25.
- (191) Gromski, P. S.; Granda, J. M.; Cronin, L. Universal Chemical Synthesis and Discovery with ‘The Chemputer’. *Trends Chem.* **2020**, *2* (1), 4-12.
- (192) Häse, F.; Roch, L. M.; Aspuru-Guzik, A. Next-Generation Experimentation with Self-Driving Laboratories. *Trends Chem.* **2019**, *1* (3), 282-291.
- (193) McCullough, K.; Williams, T.; Mingle, K.; Jamshidi, P.; Lauterbach, J. High-throughput experimentation meets artificial intelligence: a new pathway to catalyst discovery. *Phys. Chem. Chem. Phys.* **2020**, *22* (20), 11174-11196.
- (194) Coley, C. W.; Eyke, N. S.; Jensen, K. F. Autonomous Discovery in the Chemical Sciences Part I: Progress. *Angew. Chem. Int. Ed.* **2020**, *59* (51), 22858-22893.
- (195) Eyke, N. S.; Koscher, B. A.; Jensen, K. F. Toward Machine Learning-Enhanced High-Throughput Experimentation. *Trends Chem.* **2021**, *3* (2), 120-132.
- (196) Liu, Y.; Zhang, J.; Zhang, Y.; Yoon, H. Y.; Jia, X.; Roman, M.; Johnson, B. N. Accelerated Engineering of Optimized Functional Composite Hydrogels via High-Throughput Experimentation. *ACS Appl. Mater. Interfaces* **2023**, *15* (45), 52908-52920.
- (197) Park, T.; Kim, E.; Sun, J.; Kim, M.; Hong, E.; Min, K. Rapid discovery of promising materials via active learning with multi-objective optimization. *Mater. Today Commun.* **2023**, *37*, 107245.
- (198) Greenaway, R. L.; Jelfs, K. E. Integrating Computational and Experimental Workflows for Accelerated Organic Materials Discovery. *Adv. Mater.* **2021**, *33* (11), 2004831.

- (199) Wang, K.; Dowling, A. W. Bayesian optimization for chemical products and functional materials. *Curr. Opin. Chem. Eng.* **2022**, *36*, 100728.
- (200) Bai, Y.; Khoo, Z. H. J.; Made, R. I.; Xie, H.; Lim, C. Y. J.; Handoko, A. D.; Chellappan, V.; Cheng, J. J.; Wei, F.; Lim, Y.-F.; et al. Closed Loop Multi-Objective Optimization for Cu-Sb-S Photoelectrocatalytic Materials Discovery. *Adv. Mater.* **2023**, *n/a* (n/a), 2304269.
- (201) Di Fiore, F.; Nardelli, M.; Mainini, L. Active Learning and Bayesian Optimization: a Unified Perspective to Learn with a Goal. 2023; p arXiv:2303.01560.
- (202) Shahriari, B.; Swersky, K.; Wang, Z.; Adams, R. P.; Freitas, N. d. Taking the Human Out of the Loop: A Review of Bayesian Optimization. *Proc. IEEE* **2016**, *104* (1), 148-175.
- (203) Rasmussen, C. E.; Williams, C. K. I. *Gaussian Processes for Machine Learning*; The MIT Press, 2005. DOI: 10.7551/mitpress/3206.001.0001.
- (204) Chen, H.; Zheng, L.; Kontar, R. A.; Raskutti, G. Gaussian process parameter estimation using mini-batch stochastic gradient descent: convergence guarantees and empirical benefits. *J. Mach. Learn. Res.* **2022**, *23* (1), Article 227.
- (205) Burger, B.; Maffettone, P. M.; Gusev, V. V.; Aitchison, C. M.; Bai, Y.; Wang, X.; Li, X.; Alston, B. M.; Li, B.; Clowes, R.; et al. A mobile robotic chemist. *Nature* **2020**, *583* (7815), 237-241.
- (206) Shields, B. J.; Stevens, J.; Li, J.; Parasram, M.; Damani, F.; Alvarado, J. I. M.; Janey, J. M.; Adams, R. P.; Doyle, A. G. Bayesian reaction optimization as a tool for chemical synthesis. *Nature* **2021**, *590* (7844), 89-96.
- (207) Settles, B. *Active Learning*; Morgan & Claypool Publishers, 2012.

- (208) Kusne, A. G.; Yu, H.; Wu, C.; Zhang, H.; Hattrick-Simpers, J.; DeCost, B.; Sarker, S.; Oses, C.; Toher, C.; Curtarolo, S.; et al. On-the-fly closed-loop materials discovery via Bayesian active learning. *Nat. Commun.* **2020**, *11* (1), 5966.
- (209) Min, K.; Cho, E. Accelerated Discovery of Novel Inorganic Materials with Desired Properties Using Active Learning. *J. Phys. Chem. C* **2020**, *124* (27), 14759-14767.
- (210) Bassman Oftelie, L.; Rajak, P.; Kalia, R. K.; Nakano, A.; Sha, F.; Sun, J.; Singh, D. J.; Aykol, M.; Huck, P.; Persson, K.; et al. Active learning for accelerated design of layered materials. *npj Comput. Mater.* **2018**, *4* (1), 74.
- (211) Kusne, A. G.; McDannald, A. Scalable multi-agent lab framework for lab optimization. *Matter* **2023**, *6* (6), 1880-1893.
- (212) Kontar, R.; Shi, N. C.; Yue, X. B.; Chung, S.; Byon, E.; Chowdhury, M.; Jin, J. H.; Kontar, W.; Masoud, N.; Nouiehed, M.; et al. The Internet of Federated Things (IoFT). *IEEE Access* **2021**, *9*, 156071-156113.
- (213) Zhai, Z.; Zhou, Y.; Korovich, A. G.; Hall, B. A.; Yoon, H. Y.; Yao, Y.; Zhang, J.; Bortner, M. J.; Roman, M.; Madsen, L. A.; et al. Synthesis and Characterization of Multi-Reducing-End Polysaccharides. *Biomacromolecules* **2023**, *24* (6), 2596-2605.
- (214) Zhou, Y.; Zhai, Z.; Yao, Y.; Stant, J. C.; Landrum, S. L.; Bortner, M. J.; Frazier, C. E.; Edgar, K. J. Oxidized hydroxypropyl cellulose/carboxymethyl chitosan hydrogels permit pH-responsive, targeted drug release. *Carbohydr. Polym.* **2023**, *300*, 120213.
- (215) Frazier, P. I. A Tutorial on Bayesian Optimization. 2018; p arXiv:1807.02811.
- (216) Gardner, J. R.; Pleiss, G.; Bindel, D.; Weinberger, K. Q.; Wilson, A. G. GPyTorch: Blackbox Matrix-Matrix Gaussian Process Inference with GPU Acceleration. In *32nd Conference on Neural*

*Information Processing Systems (NIPS)*, Montreal, CANADA, Dec 02-08, 2018; Neural Information Processing Systems (Nips): LA JOLLA, 2018; Vol. 31.

(217) Yue, X.; Al Kontar, R.; Berahas, A. S.; Liu, Y.; Zai, Z.; Edgar, K.; Johnson, B. N. Collaborative and Distributed Bayesian Optimization via Consensus: Showcasing the Power of Collaboration for Optimal Design. 2023; p arXiv:2306.14348.

(218) UMDDataScienceLab. *Consensus\_Bayesian\_Opt.* 2023.  
[https://github.com/UMDataScienceLab/Consensus\\_Bayesian\\_Opt/tree/main](https://github.com/UMDataScienceLab/Consensus_Bayesian_Opt/tree/main) (accessed.

(219) Kim, H.-L.; Jung, G.-Y.; Yoon, J.-H.; Han, J.-S.; Park, Y.-J.; Kim, D.-G.; Zhang, M.; Kim, D.-J. Preparation and characterization of nano-sized hydroxyapatite/alginate/chitosan composite scaffolds for bone tissue engineering. *Mater. Sci. Eng. C* **2015**, 54, 20-25.

(220) Liu, Q.; Li, Q.; Xu, S.; Zheng, Q.; Cao, X. Preparation and Properties of 3D Printed Alginate–Chitosan Polyion Complex Hydrogels for Tissue Engineering. In *Polymers*, 2018; Vol. 10.

(221) Yu, C.-C.; Chang, J.-J.; Lee, Y.-H.; Lin, Y.-C.; Wu, M.-H.; Yang, M.-C.; Chien, C.-T. Electrospun scaffolds composing of alginate, chitosan, collagen and hydroxyapatite for applying in bone tissue engineering. *Mater. Lett.* **2013**, 93, 133-136.

(222) Kolanthai, E.; Sindu, P. A.; Khajuria, D. K.; Veerla, S. C.; Kuppuswamy, D.; Catalani, L. H.; Mahapatra, D. R. Graphene Oxide—A Tool for the Preparation of Chemically Crosslinking Free Alginate–Chitosan–Collagen Scaffolds for Bone Tissue Engineering. *ACS Appl. Mater. Interfaces* **2018**, 10 (15), 12441-12452.

(223) Lee, K. Y.; Mooney, D. J. Alginate: Properties and biomedical applications. *Prog. Polym. Sci.* **2012**, 37 (1), 106-126.

- (224) Pellá, M. C. G.; Lima-Tenório, M. K.; Tenório-Neto, E. T.; Guilherme, M. R.; Muniz, E. C.; Rubira, A. F. Chitosan-based hydrogels: From preparation to biomedical applications. *Carbohydr. Polym.* **2018**, *196*, 233-245.
- (225) Yuan, X.; Liu, R.; Zhang, W.; Song, X.; Xu, L.; Zhao, Y.; Shang, L.; Zhang, J. Preparation of carboxymethylchitosan and alginate blend membrane for diffusion-controlled release of diclofenac diethylamine. *J. Mater. Res. Technol.* **2021**, *63*, 210-215.
- (226) Wu, P.; Fang, Y.; Chen, K.; Wu, M.; Zhang, W.; Wang, S.; Liu, D.; Gao, J.; Li, H.; Lv, J.; et al. Study of double network hydrogels based on sodium methacrylate alginate and carboxymethyl chitosan. *Eur. Polym. J.* **2023**, *194*, 112137.