

Solution blow spinning of cell-free functionalized polymer fibers for the detection of emerging biological threats

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Abstract

Cell-free protein synthesis (CFPS) is a synthetic biology platform that can be readily programmed to perform a variety of functions to benefit the warfighter simply by changing the DNA input into the system. Increasing the utility of CF systems is the ability to be lyophilized for shelf storage and rehydrated in situ for on-demand use in the field. These lyophilized CF systems are most often implemented in tubes or on matrices like paper but can be sensitive to environmental conditions such as humidity, thus their operation in the field is limited to more controlled environments. Embedding CF systems into polymers has the potential to expand their utility into more practical form factors that can be integrated into warfighter equipment to protect the systems from the elements as well as provide versatile detection capabilities. This work demonstrates the feasibility of embedding CF systems into polymer fibers where envisioned end products could include functionalized textiles, wipes, and filters. Solution blow spinning (SBS) is a scalable technique in which compressed air pulls polymer solutions into fibers. Here, SBS is used to create non-woven fiber mats from polycaprolactone (PCL), poly(vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP), and polyethylene oxide (PEO) solutions containing lyophilized cell-free reactions. Fiber properties are characterized, and cell-free activity assessed using colorimetric and fluorescent assays.

Approach

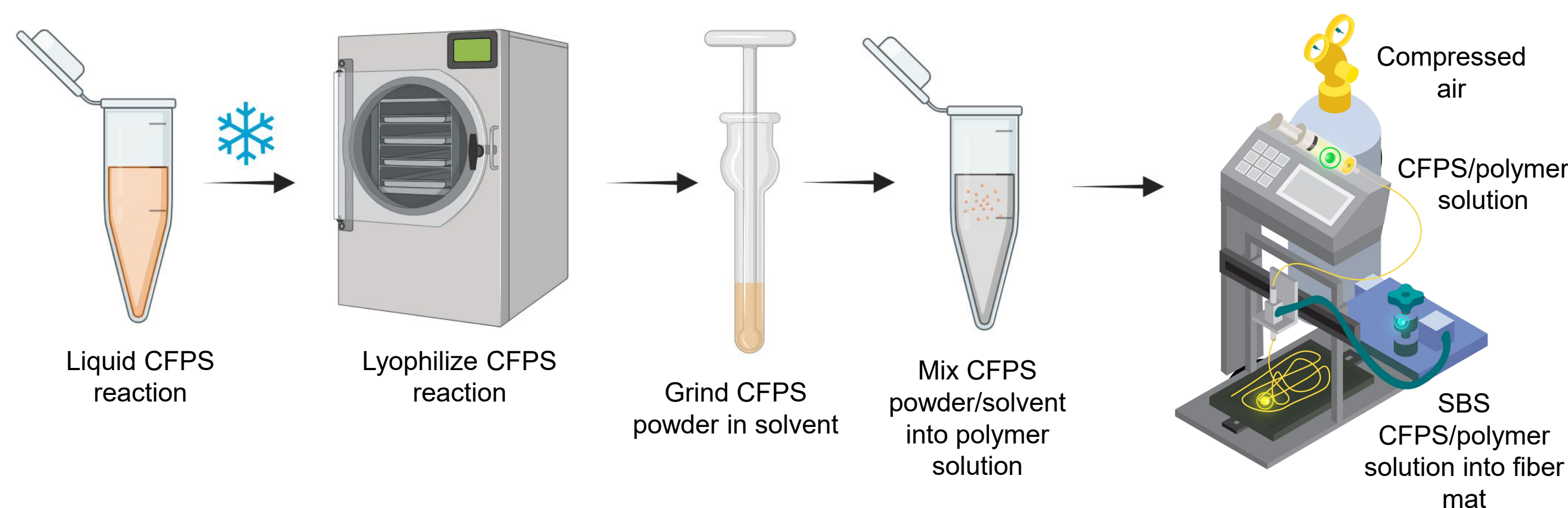


Figure 1. Solution blow spinning (SBS) of CFPS/polymer fibers. CFPS reactions are lyophilized and ground into a fine powder in solvent. The powder is mixed with polymer (1:5 by volume) to make a CFPS/polymer solution for SBS into fibers.



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Fiber Characterization

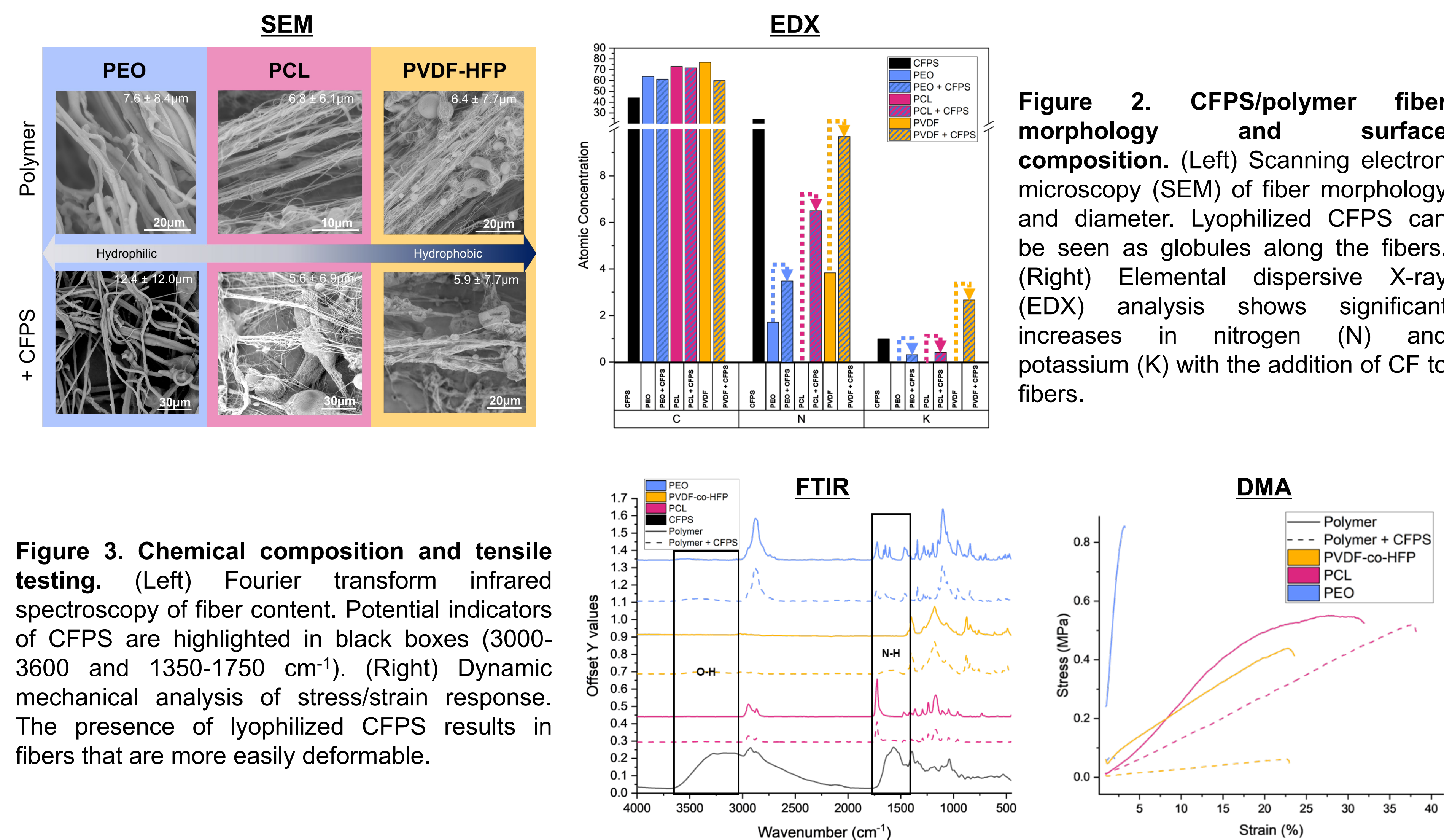


Figure 3. Chemical composition and tensile testing. (Left) Fourier transform infrared spectroscopy of fiber content. Potential indicators of CFPS are highlighted in black boxes (3000-3600 and 1350-1750 cm⁻¹). (Right) Dynamic mechanical analysis of stress/strain response. The presence of lyophilized CFPS results in fibers that are more easily deformable.

Figure 2. CFPS/polymer fiber morphology and surface composition. (Left) Scanning electron microscopy (SEM) of fiber morphology and diameter. Lyophilized CFPS can be seen as globules along the fibers. (Right) Elemental dispersive X-ray (EDX) analysis shows significant increases in nitrogen (N) and potassium (K) with the addition of CF to fibers.

NMR spectroscopy of lyophilized CFPS components

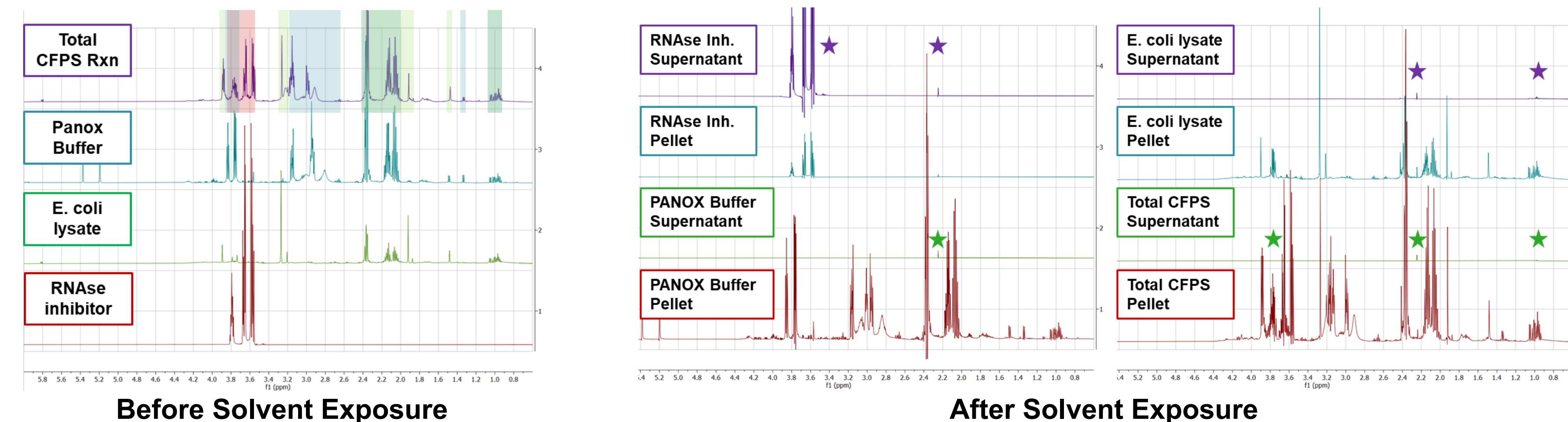


Figure 4 Nuclear magnetic spectroscopy of CFPS before and after solvent exposure. (Left) NMR spectra of lyophilized CFPS reaction components resuspended in deuterated water. (Right) NMR analysis of components exposed to acetone show most CFPS components remain insoluble during processing, except for glycerol added with RNase inhibitor. This trend was also observed after exposure with chloroform (not shown).

Cell-Free Activity and Shelf Life

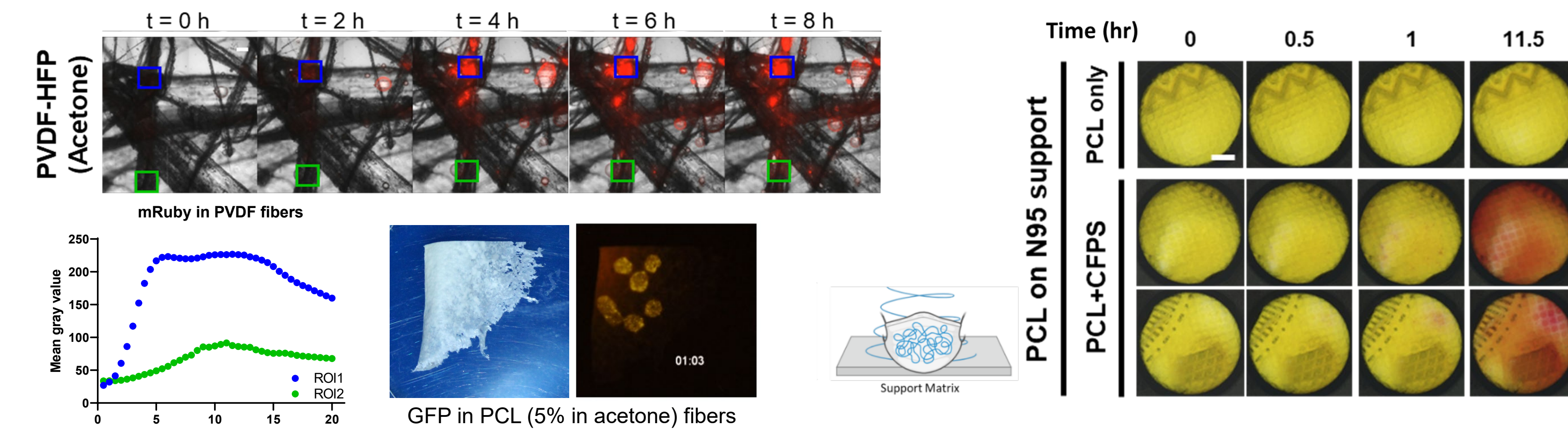


Figure 5. Cell-free activity in CFPS/polymer fibers. (Left) Microscope images of CFPS/PVDF spun fibers producing mRuby fluorescence over time. Images of sfGFP fluorescence following rehydration of a CFPS/PCL fiber mat. (Right) CFPS/PCL fibers constitutively expressing β -galactosidase (LacZ) spun onto an N95 mask and rehydrated (5mM CPRG) for a yellow to purple color change.

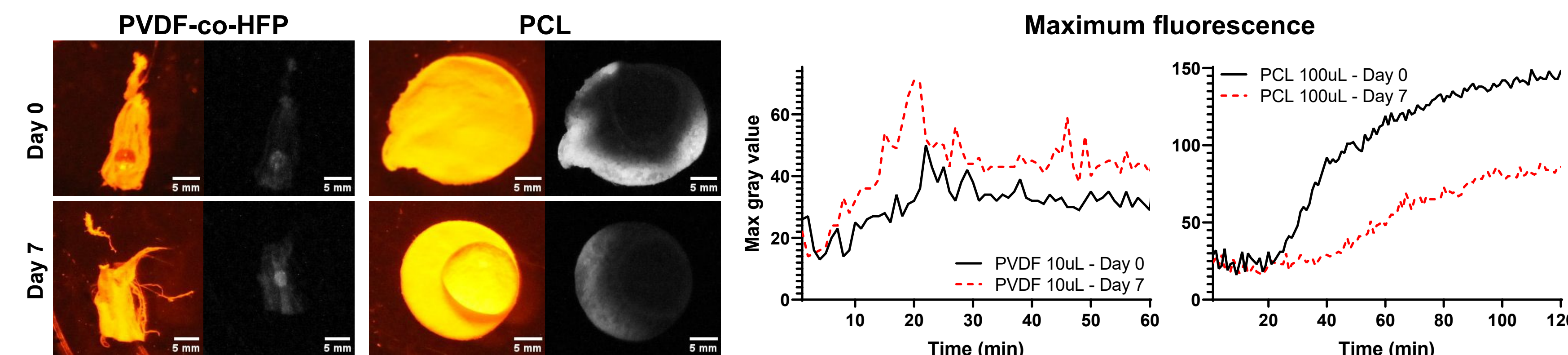


Figure 6. Shelf life of CFPS/polymer fibers. PVDF and PCL fibers exhibited CF activity after storage in a desiccator for 1 week. PVDF fibers demonstrated the same amount of fluorescence while PCL fibers lost activity compared to Day 0.

Conclusions

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- Lyophilized CFPS is not fully encapsulated in single polymer SBS fibers
- Incorporation of CFPS results in more easily deformable polymer fibers
- Lyophilized CFPS remains active after being processed into SBS polymer fibers
- CFPS/polymer fibers can maintain activity after a week of storage

Future Directions

- Explore coaxial spinning of lyophilized CFPS using incompatible solvents
- Demonstrate applications of CFPS/polymer fibers as sensors, filters, and decontamination products

Acknowledgements: Funding was provided by the U.S. Army via the Chemical Biological Advanced Materials Manufacturing Program (PE 0601102A Project VR9) at the Combat Capabilities Development Command Chemical Biological Center. The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense or the U.S. Government.)