Advanced Characterization of Engineered Living Materials

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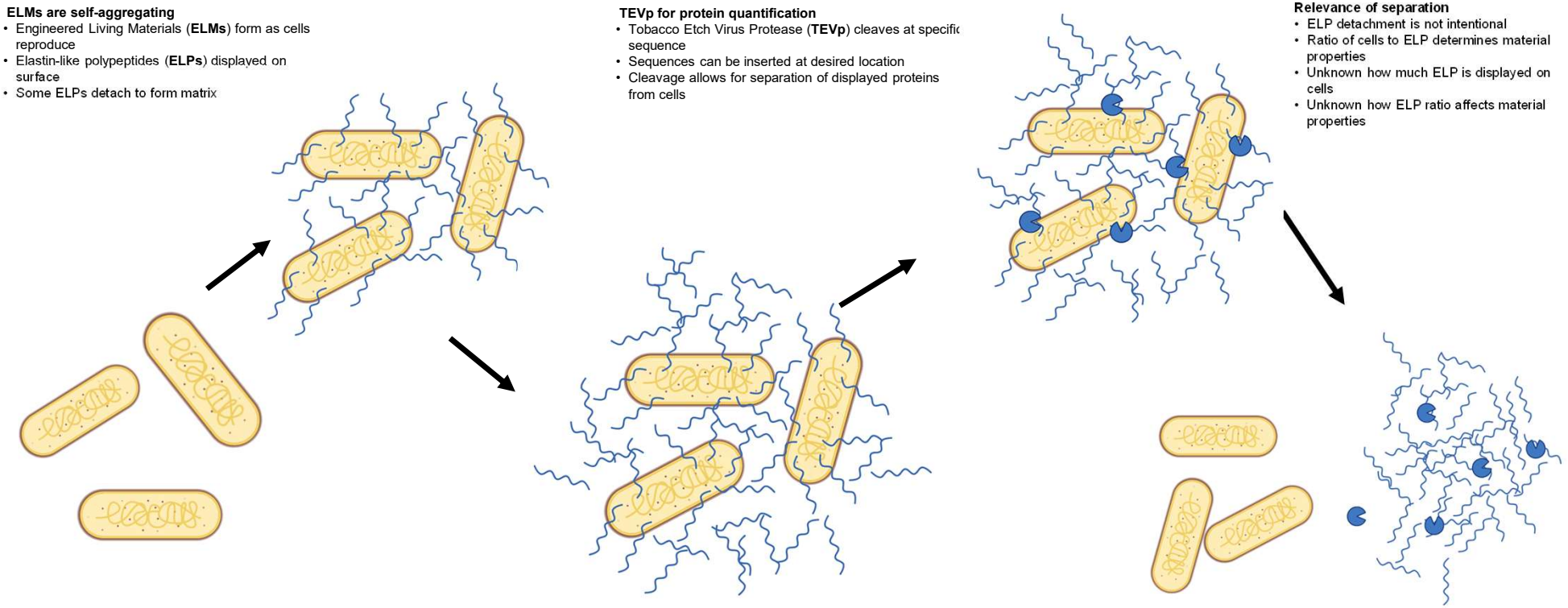
Background

ELMs are self-aggregating
- Engineered Living Materials (**ELMs**) form as cells reproduce
- Elastin-like polypeptides (**ELPs**) displayed on surface
- Some ELPs detach to form matrix

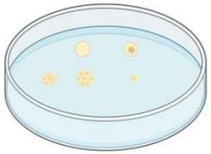
TEVp for protein quantification
- Tobacco Etch Virus Protease (**TEVp**) cleaves at specific sequence
- Sequences can be inserted at desired location
- Cleavage allows for separation of displayed proteins from cells

Relevance of separation
- ELP detachment is not intentional
- Ratio of cells to ELP determines material properties
- Unknown how much ELP is displayed on cells
- Unknown how ELP ratio affects material properties



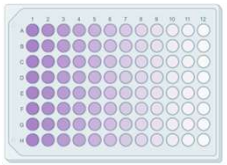
Methods

Colony Forming Unit (CFU) Counting



Addition of TEVp

BCA Assay



Western Blot

Results

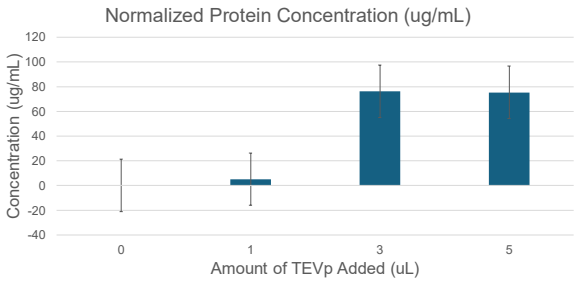


Fig 1. BCA assay results Normalized protein concentrations obtained for samples containing 0, 1, 3, or 5 microliters of TEVp added to the resuspended cells. Samples were normalized by subtracting BCA values of TEVp controls, which were samples containing protease but no cells. Error bars present show standard error. The data show addition of TEVp clearly increases the concentration of protein present in the sample, and normalization demonstrates a significant portion of this increase is not due to the presence of TEVp in the sample. Taken together, this suggests ELP is in fact cleaved from the cell surface via the TEVp mechanism.

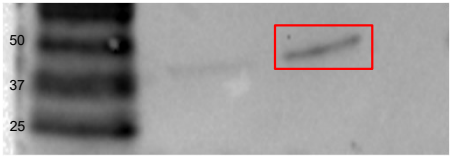


Fig 2. Western Blot of BCA samples
Western Blot immunoblotting assays were performed to analyze the protein content obtained from BCA assay testing. Reference kDa numbers are labeled on the ladder. The ELP band appears at 46 kDa (highlighted in red). Proteins were isolated using a SpyTag sequence present in the ELP plasmid.

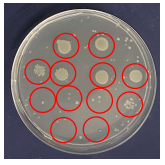


Fig 3. CFU analysis
Dilutions of cell culture were plated, grown overnight, and counted to quantify the number of colony-forming units per milliliter of cells (cluster locations circled in red). This was used to establish the ratio of protein (ELP) to cells (CFU). This ratio was determined to be 2.37×10^{-6} ug of ELP per CFU

Summary and Future Directions

- I demonstrated that TEVp can be used to cleave proteins from cell surfaces.
- The presence of more protein in the supernatant than in the resuspended cell mixture or the TEVp control suggests ELP is present, confirmed by the results of the Western Blot.
- Using a combination of techniques to quantify the cells and protein in a sample, I determined the ratio of cells to protein.
- Next steps include testing to determine how cleavage of ELP from the cell surface affects material formation.

References

1. Molinari et al. *Nature Communications* 2022
2. Cesaratto et. al. *Journal of Biotechnology* 2016
Images created using BioRender
Graphs created in Microsoft EXCEL

Further Information

Please feel free to reach out to us via email, sander26@terpmail.umd.edu,
or check out our website using the QR Code.