Tunable Biocomposite Dextran-ELP and Dextran-RLP Hydrogels as Smart Biomaterials

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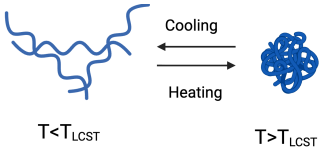
Introduction

- Smart Biomaterials are biocompatible constructs that are able to dynamically respond to stimuli
- Thermoresponsive polymers react to changes in environmental temperature causing structural changes
- Dextran Methacrylate is a carbohydrate based polymer that possesses a temperature phase separation property
- Elastin-like polypeptide and Resilin-like-polypeptide are thermoresponsive polypeptides derived from human Elastin and insect resilin respectively.
- Here multiple biocomposite materials of Dextran-ELP and Dextran RLP are proposed



Objectives

- Synthesize 2 novel thermo-responsive biomaterials between a polysaccharide and 2 proteins.
- Analyze rheological properties and test material for wound healing and drug delivery applications
- Analyzing temperature dependent phase transition behavior on the composite materials
- Characterize the small supramolecular structure of the temperature dependent biocondensates

Methods

RLP

HHHHHH-(GGK)$_3$-**(RES)**-(GGK)$_3$-**(RES)**-(GGK)$_3$-**(RES)**-(GGK)$_3$-**(RES)**-(GGK)$_3$-(Stop)

ELP

HHHHHH-(GGK)$_3$-**(VPGIG)**$_{25}$-(GGK)$_3$-**(VPGIG)**$_{25}$-(GGK)$_3$-**(VPGIG)**$_{25}$-**(VPGIG)**$_{25}$-(GGK)$_3$-**(VPGIG)**$_{25}$-(GGK)$_3$-(Stop)

Fig 1. Expression plasmid for ELP/RLP constructs. *(RES)=GGRPSDSFGAPGGGN

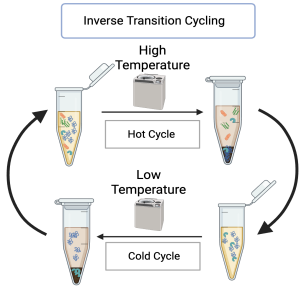


Fig 2. diagram of inverse transition cycling for non-chromatography based purification of ELP/RLP from cell lysates.

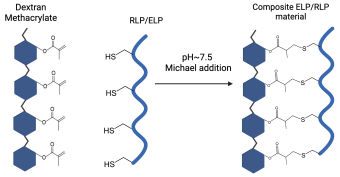


Fig 3. Conjugation of Dextran Methacrylate to RLP/ELP polypeptide with lysin functional groups

Results

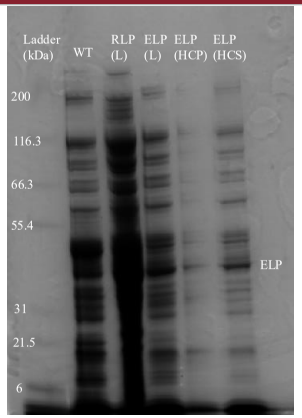


Fig 4. SDS-PAGE of ELP through multiple stages of ITC cycle. (Ladder) Mark 12 Invitrogen standard, (WT) wild-type BL21 E.coli, (RLP L) Resilin-like Polypeptide Lysate, (ELP L) Elastin-like polypeptide Lysate, (ELP HCP) Elastin-like polypeptide pellet composition after 1st hot cycle, (ELP HCS) Elastin-like polypeptide supernatant after 1st hot cycle

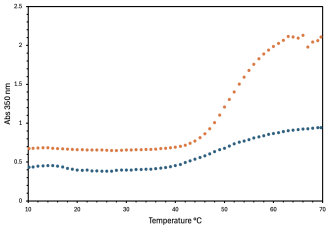


Fig 5. Turbidity analysis of protein extracts across temperature range 10C-70C. (Orange) protein supernatant retrieved after hot cycle (Orange) and pelleted protein from hot cycle (Blue).

Conclusions

- Significant losses in desired ELP product occur during 1st round of ITC during hot cycle
- The examined ELP construct presented a lower LCST temperature than would be expected for its size and composition.
- Evaluation of the of the current ELP construct against similarly composed and previously characterized ELP may provide data for comparison

Future Plan

- Troubleshoot ITC purification process for low yields
- Use the High Purity ELP product for Dex-ELP synthesis
- Analyze Dex-ELP conjugate with Rheometer for mechanical properties
- Purify RLP with affinity chromatography

Acknowledgement

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