Biomaterials Research Projects for Undergraduate Students: Exploring the Correlation between Silk Fibroin Protein Aggregation and Intrinsic Fluorescence

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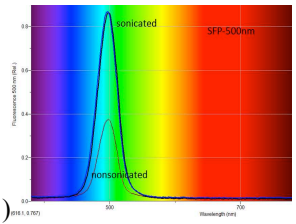
Statement of Purpose: This study describes a research project that was carried out as part of an independent study course in biology under the supervision of two faculty members with biochemistry and biomaterials expertise. The project was specifically designed in collaboration with an undergraduate biomedical engineering student for future implementation as part of interdisciplinary sciences courses for engineering students. Prior to this course, the student had taken 12 credits of chemistry and 16 credits of biology but had not taken any courses on biomaterials. The purpose of incorporating this project into the course was to introduce biomaterials and their role in creating new tools for the detection and diagnosis of diseases. Silk fibroin protein (SFB) was specifically chosen for this purpose. SFB is a widely studied protein for a variety of biomedical applications, mainly due to its suitable mechanical properties, biocompatibility and controlled proteolytic degradability.[1]

This project was designed to first introduce several biomaterials fabrication methods: SFP scaffolds, SFP hydrogels and insoluble SFP films for biomedical applications. The student was then instructed to make SFP hydrogels by different methods and investigate the correlation between intrinsic fluorescence and protein aggregation as a model for non-invasive detection of neurodegenerative diseases. A variety of neurodegenerative disorders such as Alzheimer's are characterized by the formation of amyloid fibril aggregates. Several studies suggest that detection of amyloid aggregation, associated with intrinsic fluorescence of this protein, can be used for an early detection of this disease.[2] Thus, in this study, SFP and enhanced green fluorescence protein (eGFP) were used as model proteins to study the correlation between protein aggregation and intrinsic fluorescence.

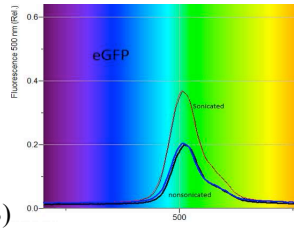
Methods: Liquid SFP and eGFP (expressed in bacteria) were obtained from Silktap Inc. and Edvotek Inc., respectively. As the first part of the project, student created SFP scaffolds (using sodium chloride porogens), 2D non-soluble SFP films (using methanol treatment) and SFP hydrogels (using three methods: sonication, vortexing and pH change). In the second part of the project, ultrasonication of SFP and eGFP was carried out using Fisher Scientific Model 705 Sonic Dismembrator (titanium1/8 micro-tip). The sonication procedure was first optimized using various gelation times and amplitudes on either a single or a binary protein solution. Samples, sonicated at 20% amplitude for 45 seconds, were then analyzed using a fluorometer (Vernier SpectroVis Plus Fluorometer) at 500nm Wavelengths. Sonicated samples were compared with their non-sonicated protein samples.

Results: Various SFP constructs were successfully fabricated in the lab during the semester. Based on the optimized ultrasonication parameters, hydrogels of SFP and eGFP were made and their intrinsic fluorescence was measured. As shown in Figure 1 (A and B), a single solution of eGFP and SFP showed increased fluorescence at 500nm wavelength after gelation by sonication. A decrease in fluorescence, however, was observed when a binary protein solution of eGFP and SFP (1:1 ratio) was sonicated compared with a pre-sonicated solution (Fig1, C). All samples containing eGFP emitted green fluorescence post-sonication.

A)



B)



C)

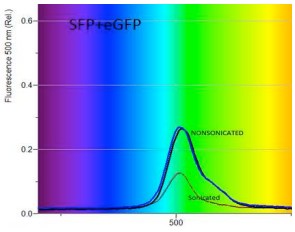


Figure 1. Intrinsic fluorescence measured before and after sonication at 500nm wavelength. A) SFP; B) eGFP aC) SFP+eGFP (1:1 ratio)

Conclusions: A project, designed in collaboration with an undergraduate biomedical engineering student, was successfully carried out and completed over one semester. In this project, various biomaterials fabrication techniques were introduced. Additionally, the student studied the correlation between protein aggregation and intrinsic fluorescence and its potential use for the diagnosis of neurodegenerative diseases. The results showed that the intrinsic fluorescence of SFP and eGFP model proteins changes due to protein aggregation and that single and binary protein solutions behave differently in this regard. This project has currently been extended and the student is now investigating the aggregation of beta-amyloid peptide and its potential in early detection of neurodegenerative diseases such as Alzheimer's.

References:

1. Vepari C and Kaplan DL. Porg. Polym Sci. 2007; 32: 991-1007.
2. Chan FT et al. R. Soc. Chem. 2013; 7: 2156-2162.