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Effects of Polyphenols on Tropoelastin Assembly

Helena Teixeira-Dasilva
Washington University in St. Louis

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Washington University in St. Louis
School of Engineering & Applied Science

Helena Teixeira-Dasilva, research under Jessica E. Wagenseil

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Abstract

The purpose of this study was to understand the effect of epigallocatechin gallate (EGCG) on the coacervation process of tropoelastin. The initial trials indicate that coacervation rate and intensity increase with additional EGCG supplement. Results of this study can guide research towards a regenerative solution to aortic aneurysms.

Keywords:

Aneurysm, elastin, coacervation, elastin soluble 12 (ES12), epigallocatechin gallate (EGCG)

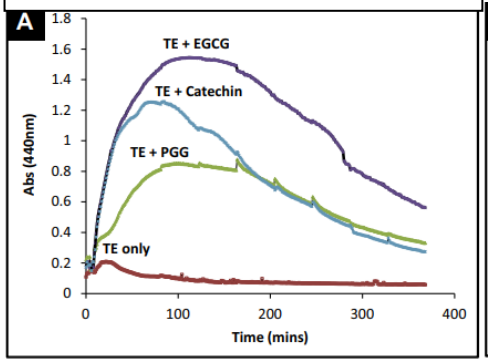
Introduction

Elastin is a critical elastic fiber responsible for the structure and resilience of tissue in the body. When an aortic aneurysm occurs, elastin fibers are damaged, disrupting the essential function of the aorta in the heart. The question of aneurysm prevention is readily researched and preventative measures such as surgery are used in practice. However, methods to repair damaged elastin in the case of aneurysm development are largely unknown. To replicate and reform elastic tissue, it is necessary to form elastin at rapid rates and prevent its degradation. One theory is that naturally occurring polyphenols can act as catalysts in the formation process and slow the degradation of the elastic fibers once formed [1].

The formation of elastin involves the aggregation of tropoelastin protein molecules in a process called coacervation. Coacervation is temperature and pH dependent and is the result of hydrophobic interactions between tropoelastin molecules [2]. Polyphenols are characterized by having multiple hydroxyl groups and have potential influence on the kinetics of the coacervation process. Two such polyphenols are: pentagalloyl glucose (PGG) and epigallocatechin gallate (EGCG). This study focuses on the coacervation, in relation to the absorbance of the solution, in tropoelastin solutions supplemented with varying PGG and EGCG concentrations.

Previous work has been done in the Wagenseil lab and other labs to study the effect of polyphenols on tropoelastin assembly. The Wagenseil lab's previous experiments indicated that using ES12 for the tropoelastin solution lead to more stable results than alternatives. Additionally, the Wagenseil lab was able to produce results where coacervation rate and rate of decay of ES12 increased with PGG concentration [3]. Sinha (2014) was able to monitor the coacervation process using a UV-Vis Spectrometer with polyphenol supplements. In these experiments, the "coacervation phase" was characterized by a sharp increase in absorbance and the "maturation phase"

Fig. 1. "Self-assembly and coacervation kinetics of tropoelastin after polyphenols addition as studied by UV spectroscopy" [1].



occurred during the steady absorbance decrease after [1]. The objective of this study was to replicate the results for EGCG at multiple concentrations of elastin and polyphenol and compare the results.

Procedure

The procedure was modified from previous work in the Wagensiel Lab. The goal was to observe absorbance levels of twelve different combinations of ES12 and EGCG concentrations over a two hour period. The following procedures were used to develop the essential materials for the experiment.

Acetic Acid Buffer modified from [3]:
[1] Obtain 80mL of distilled water.
[2] Measure and add 0.5772g of Sodium Acetate to the distilled water solution.
[3] Add 186 uL of acetic acid into the solution.
[4] Adjust the pH of the solution to be 4.6 using a 1N Hydrochloric acid solution and 1N Sodium hydroxide solution.
[5] Swirl and wait in between measurements to ensure the highest accuracy.
[6] Add distilled water until the total volume is 100mL.

10mL ES12 Stock solution prep modified from [3]:
[1] Obtain 10 mL of distilled water.
[2] Measure and add 0.06 g of Elastin Soluble 12 powder.
[3] Before every use vortex for 30-60 seconds.
[4] Store in 0.5-1mL aliquots in the -4C freezer depending on expected use.

EGCG 5mg/mL concentration stock (0) Prep:
EGCG Cas: 989-51-5
[1] Obtain 10mL of distilled water.
[2] Mix the distilled water with the 50mg powder of EGCG.
[3] Vortex until fully dissolved.
[4] Aliquot into 1mL and 20uL volume amounts to be stored in the -4C freezer.

EGCG Experiment Set-Up:

Once the materials were prepared, the experiment could be run according to the following procedure:

[1] Measure the buffer pH and adjust to 4.6 if necessary.
[2] Place the well plate and pipette tips into the freezer for cooling.
[3] Remove and thaw two 1mL ES12 stock ($\frac{6\ mg\ ES12}{mL\ H_2O}$) and two 20uL EGCG stock(0) ($\frac{5\ mg\ EGCG}{mL\ H_2O}$) aliquots.
[4] Set up the spectrometer:
37°C; shaker feature - ON; wavelength - 440nm; create new plate reader from the template.
[5] Dilute stock (0) 1:10 with 4.6 Acetic Acid Buffer. Each 20uL of EGCG stock(0) should be diluted in 180uL of buffer and will need to be vortexed until solutions have clearly mixed. Then mix further by pipette mixing the solution at least 6 times.
[6] Acquire an ice bucket and place the cooled well plate, the buffer, the ES12 and EGCG solution into it.
[7] Using the cooled tips, pipette the buffer solution, then the EGCG solution according to the amounts shown in Fig. 2.
[8] After ensuring that the spectrometer is ready, add the ES12 according to Fig. 2.

[9] Immediately insert the plate into the spectrometer when completed with adding the ES12.
[10] Run the spectrometer for two hours and return to save results.

	1	2	3	4	5	6	7	8	9	10	11	12
A	30, 10, 60	30, 10,60	30,10,60	30,7,63	30,7,63	30,7,63	30, 4,66	30, 4,66	30, 4,66	30,1, 69	30,1,69	30,1,69
B	30,10,60	30,10,60	30,10,60									
C	60, 10, 30	60, 10, 30	60, 10, 30	60,7,33	60,7,33	60,7,33	60, 4,36	60, 4,36	60, 4,36	60,1, 39	60,1, 39	60,1, 39
D	60,10, 30	60,10, 30	60,10, 30									
E	90, 10, 0	90, 10, 0	90, 10, 0	90,7,3	90,7,3	90,7,3	90, 4,6	90, 4,6	90, 4,6	90,1, 9	90,1, 9	90,1, 9
F	90,10, 0	90,10, 0	90,10, 0									
G												
H												

Fig. 2. Well plate set up for EGCG experiment. Format (in mL) : (ES12, EGCG, BUFFER) and (ES12, BUFFER, BUFFER) for control rows B, D and F.

Results and Discussion

The normalized data from three trials of the experiment were used to create plots of the absorbance measured by the spectrometer over 2 hours for each concentration of ES12 used: 30uM,

Fig. 3. Average over three normalized trials with 30 uM ES12 . Error bars show standard deviation.

60uM and 90uM. Each plot has five different EGCG amounts in uL shown.

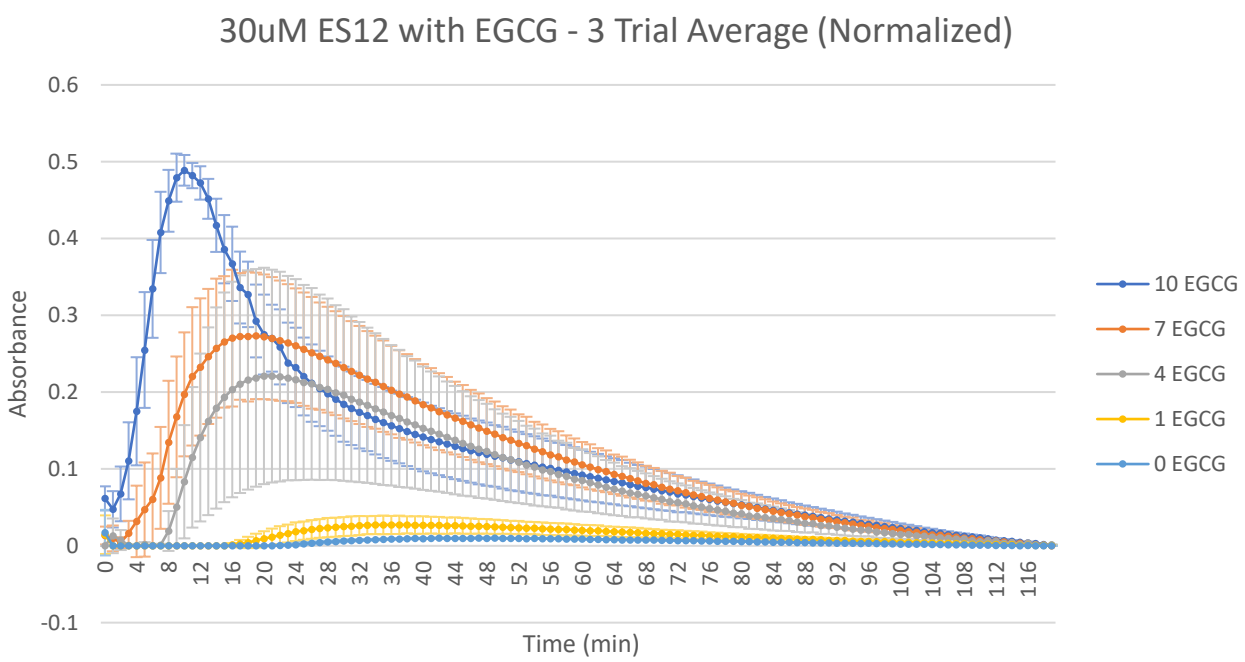

30uM ES12 with EGCG - 3 Trial Average (Normalized)
Absorbance
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96 100 104 108 112 116
Time (min)
10 EGCG
7 EGCG
4 EGCG
1 EGCG
0 EGCG

Fig. 4. Average over three normalized trials with 60 uM ES12 . Error bars show standard deviation.

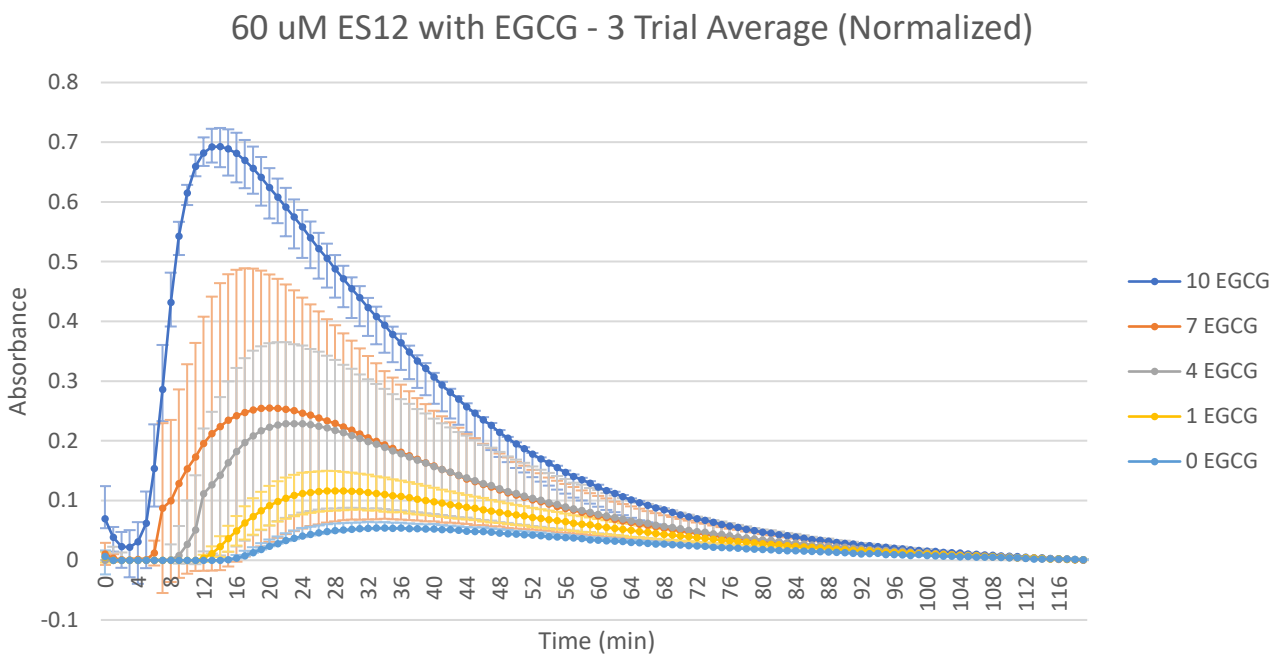
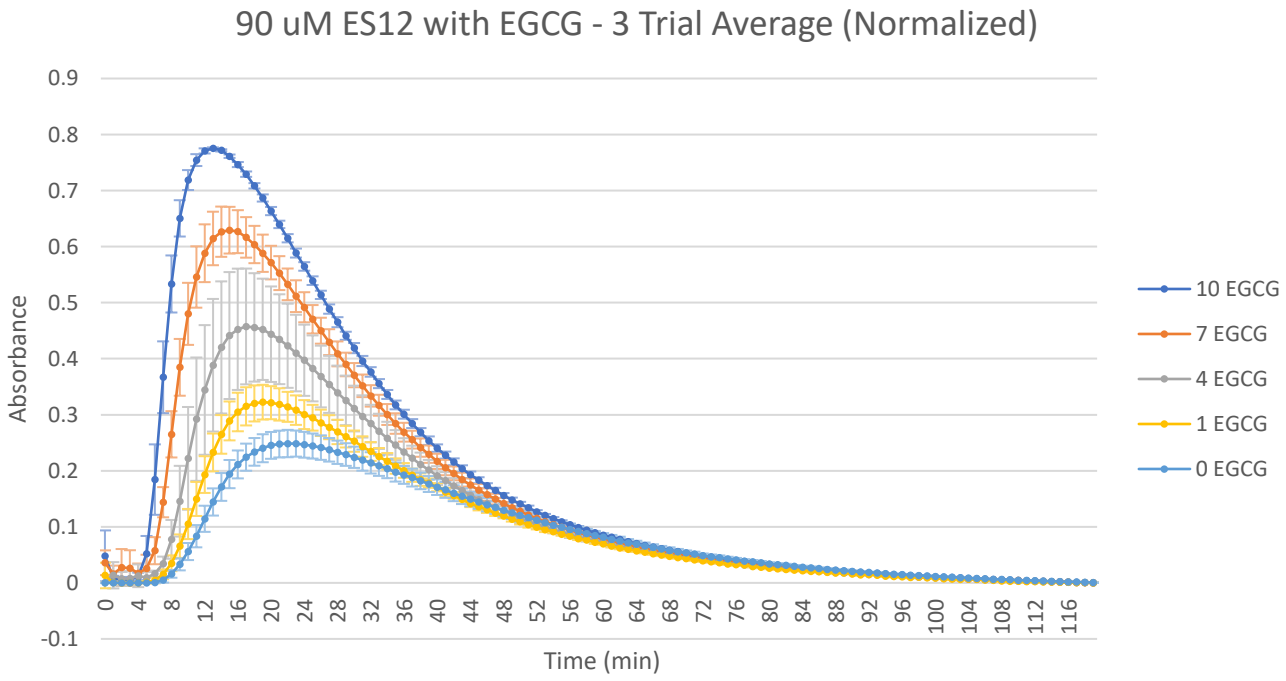
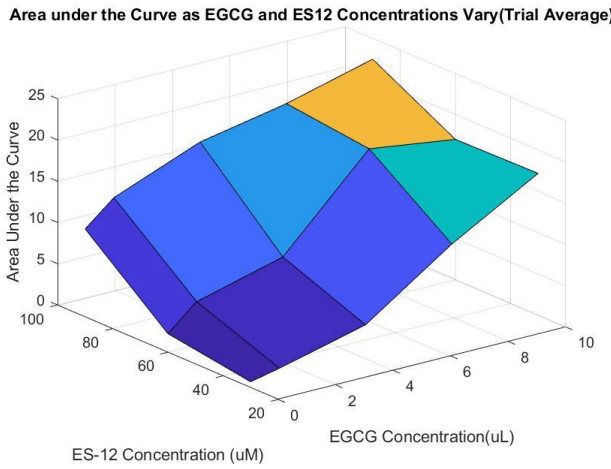


Fig. 5. Average over three normalized trials with 90 uM ES12 . Error bars show standard deviation.

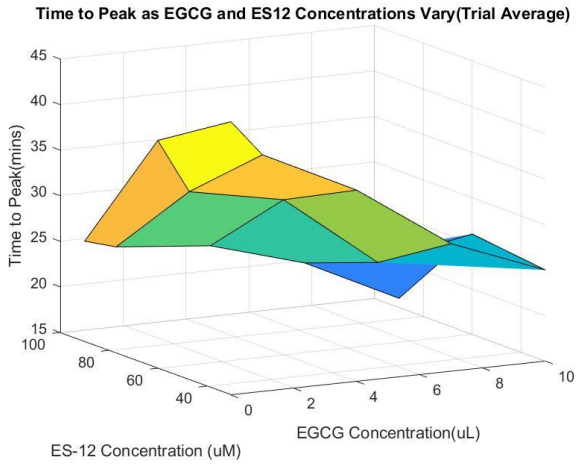


The plot of 30 uM ES12 solution (Fig. 3) indicates that 10 uL EGCG supplement leads to a significant increase in coacervation rate. The rate of decay also seems to be the highest with the 10 EGCG supplement. The 7 uL and 4 uL supplements show similar trends when considering error bars, with both coacervating and decaying slower. The 1 EGCG and 0 EGCG show little absorbance change. A similar trend is shown in the 60 uM ES12 plot (Fig. 4) with the 10 EGCG having a significantly higher and sooner peak. The 90 uM plot shows a much more even distribution. The initial coacervation rate increases proportionally to the amount of EGCG added to the solution, as does the peak absorbance. The "maturation phase" in the 90 uM plot shows a decreasing decay rate with decreasing EGCG amounts, until they converge after an hour.

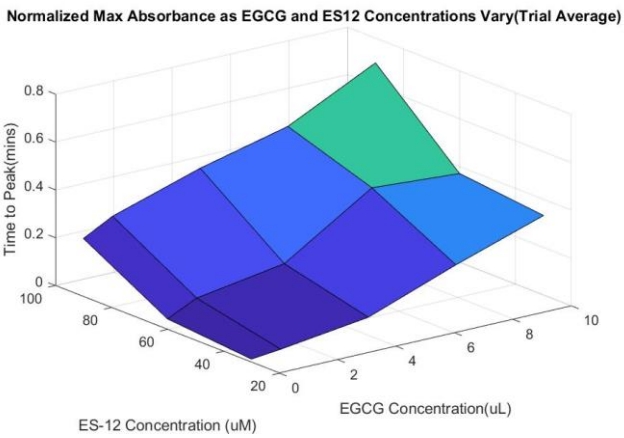
Fig. 6. MATLAB generated plots of changes with varying EGCG and ES12 concentrations



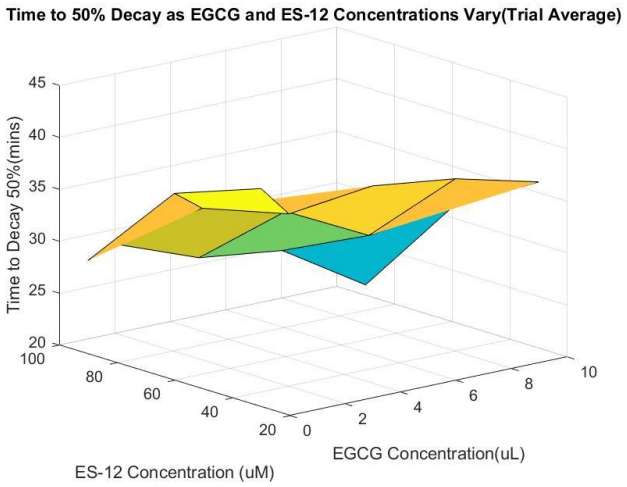
(a) Area under the curve



(b) Time to reach peak absorbance



(c) Normalized maximum absorbance value



(d) Time to reach 50% decay

Figure 6 shows how four variables representative of coacervation rate change with concentration of ES12 and EGCG. The area under the curve in Fig. 6a represents the total coacervation process. A larger area indicates a high peak and a longer overall process, which occurs at high concentrations of ES12 and EGCG. Figure 6b shows the time it takes to peak. EGCG appears to significantly decrease the time to peak. At 30 uM, the sample with no EGCG takes 45 minutes to peak while the sample with 10 uL EGCG takes only 25 minutes. The difference is less extreme at higher ES12 concentrations, but the samples with more EGCG consistently take less time to reach their peak. The maximum absorbance (Fig. 6c) increases with both concentrations, indicating that the intensity of coacervation is the highest where more EGCG and ES12 is present. The decay rate is also significantly affected by EGCG. Figure 6d shows rapid increase in decay time when more EGCG is present, with the most extreme example being 30 uM ES12.

Conclusion

The results of the experiment indicate that supplementing EGCG increases the rate of coacervation for ES12 at 30 uM, 60 uM and 90 uM concentrations. Effects of EGCG were most significant on the 30 uM ES12 samples with the control showing little to no coacervation and the 10 EGCG supplemented sample reaching a high peak absorbance early on, indicating high coacervation intensity. The most stable results were found in the 90 uM ES12 samples, indicated by smaller error bars and consistent increase in coacervation with EGCG amount. More trials should be conducted to confirm these observations. Additionally, further studies should confirm the exact correlation between absorbance and the coacervation process by imaging the experiment as it coacervates.

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References

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