

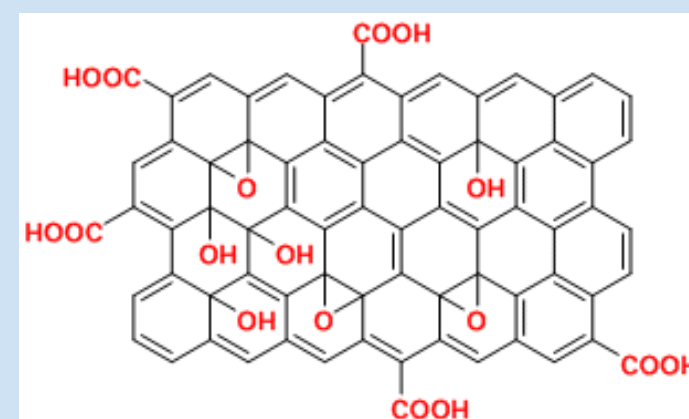
Introduction

- According to a 2018 study, approximately 60,000 Americans die each year from blood loss.
- Many deaths from hemorrhaging happen because natural blood clots form too slowly and are too weak.
- Effective promoters of blood clotting strength are vital and could save lives.
- Strong blood clotting agents could help surgeons minimize patient blood loss during surgical procedures..

Idea



Partially Reduced Graphene Oxide (pRGO) has been shown to enhance the crosslinking of gelatin by the enzyme microbial transglutaminase.



Graphene Oxide (tcichemicals.com)

Will pRGO enhance the activity of Thrombin, thereby enhancing hemostasis?

Hypothesis

Partially Reduced Graphene Oxide enhances the effect of the clotting of fibrinogen by the enzyme thrombin (an easily quantifiable reaction).

Materials and Methods

- Synthesized GO using Hummer method³
- Dissolved GO in water, 1 mg/mL, sonicated, then centrifuged to remove precipitate
- Reduced to pRGO by adding 12mM NaBH₄, then stirred for 24 hours
- Created fibrinogen solution, concentration of 10 mg/mL
- Added pRGO in varying concentrations to 8μ thrombin in 3ml fibrinogen solution
- Used a Malverne Bohlin Gemini HR Nano Rheometer to measure elastic modulus of the resulting clot over time
 - 10% strain at 37 C, time step 2 seconds



Fibrinogen clot sticking to rheometer

Enhancing Thrombin Activation of Fibrinogen Clots Using Partially Reduced Graphene Oxide

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Results

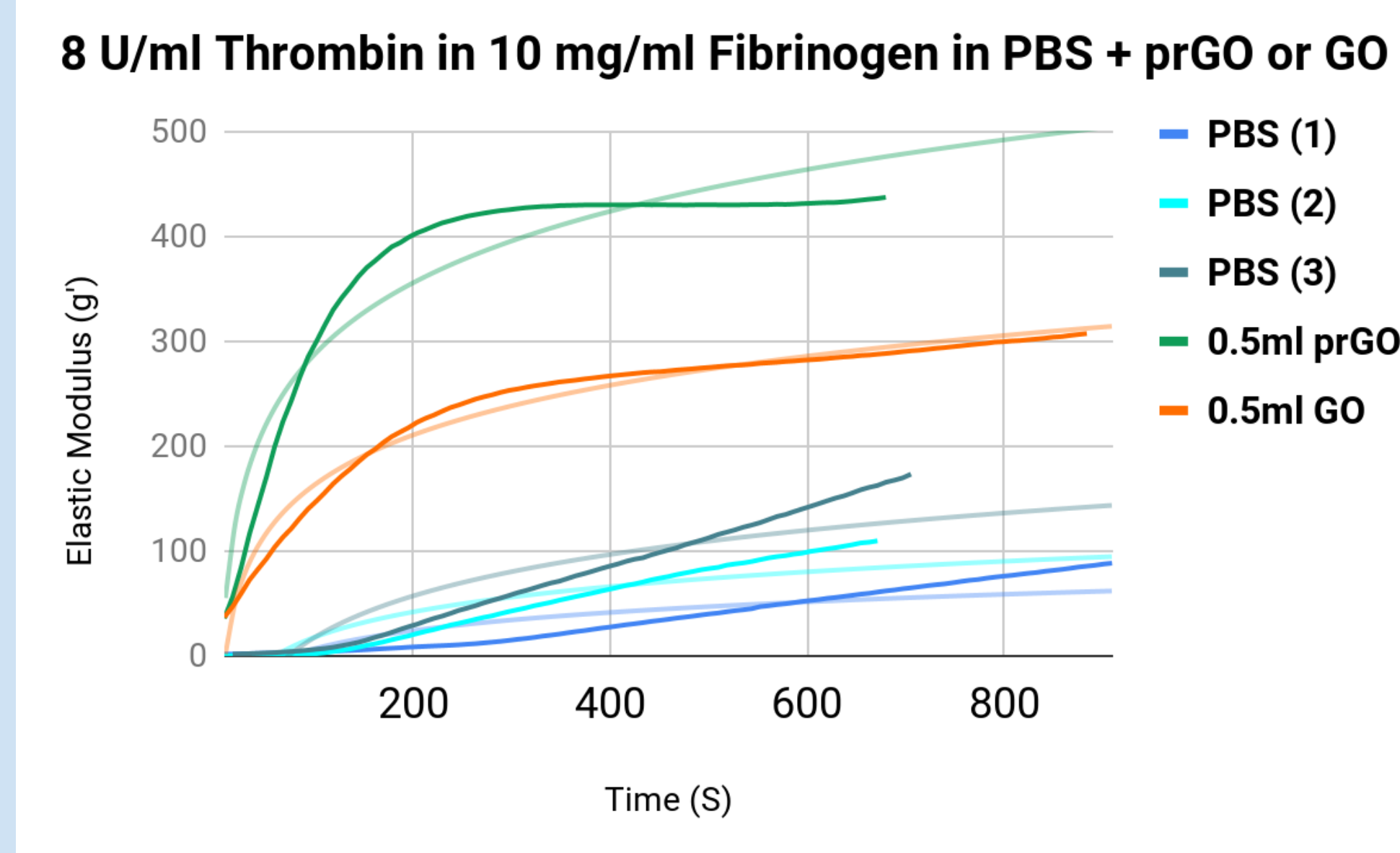


Fig 1. The pRGO solution produced a clot with an elastic modulus four times greater than that of the controls, and 1.5 times greater than that of the GO.

Solution	R ²	Trend line	Slope of rise
PBS (1)	0.687	24.9 ln x	0.095
PBS (2)	0.754	34.8 ln x	0.224
PBS (3)	0.785	57 ln x	0.292
0.5mL pRGO	0.888	98.3 ln x	1.77
0.5mL GO	0.971	68.4 ln x	0.989

Fig 3. The elastic moduli were found to be logarithmic, as the logarithmic trendlines had relatively high R² values. pRGO had the highest coefficient before the logarithm, indicating a shorter clotting time.

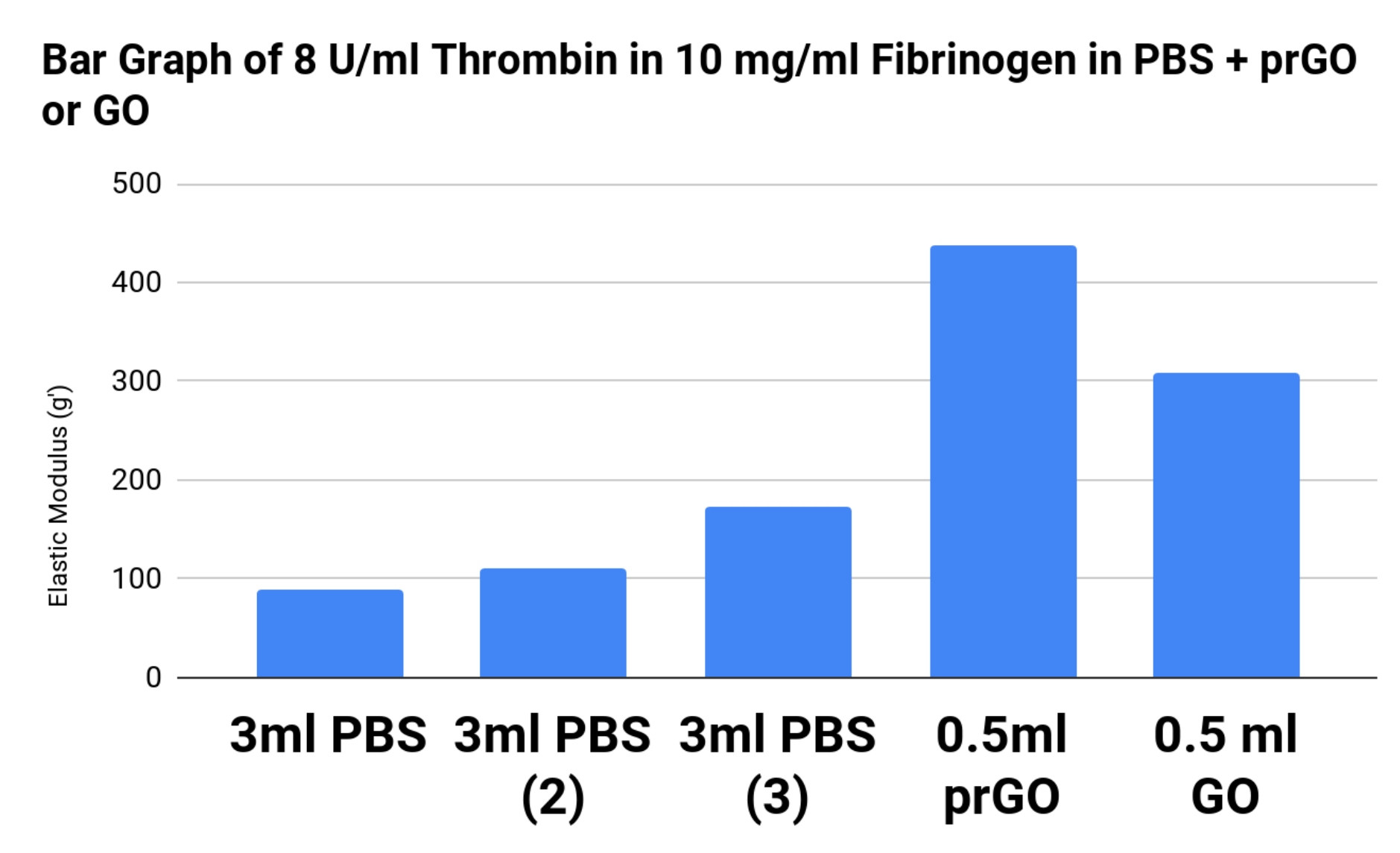


Fig 5. pRGO had the highest elastic modulus, indicating that it was the most effective at enhancing thrombin's activity

Solution	Elastic Modulus
3ml PBS	88.51
3ml PBS (2)	109.8
3ml PBS (3)	173.4
0.5ml prGO	437.4
0.5 ml GO	307.5

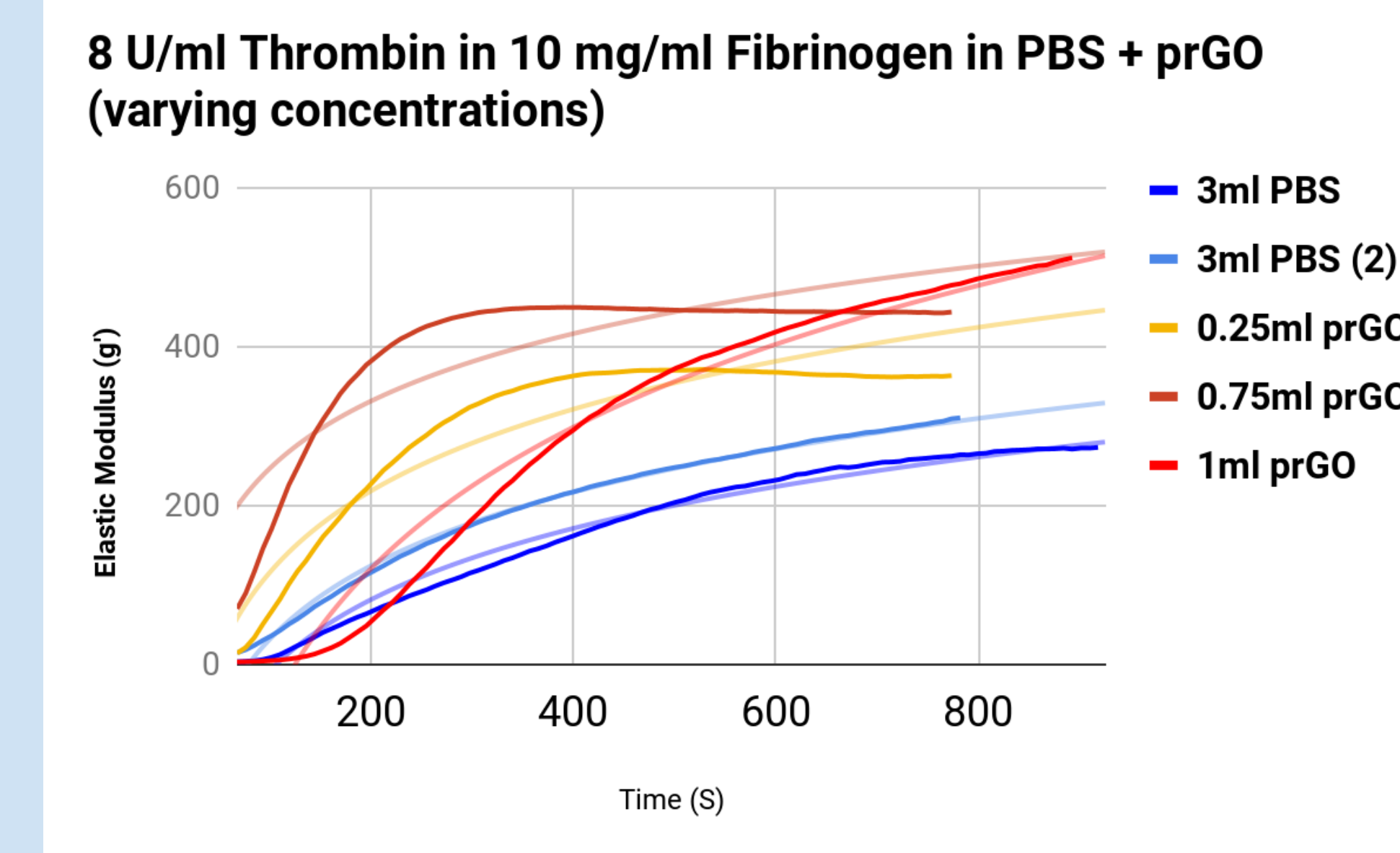


Fig 2. Increasing the concentration of pRGO led to an increase in maximum elastic modulus and to a decrease in clotting time

Solution	R ²	Trend line	Slope of rise
PBS (1)	0.976	130 ln x	0.457
PBS (2)	0.955	134 ln x	0.434
0.25mL pRGO	0.873	149 ln x	1.25
0.75mL pRGO	0.73	123 ln x	2.17
1mL pRGO	0.952	258 ln x	0.908

Fig 4. The elastic moduli were found to be logarithmic, as the logarithmic trendlines had relatively high R² values. Higher concentrations of pRGO had higher coefficients, indicating a shorter clotting time.

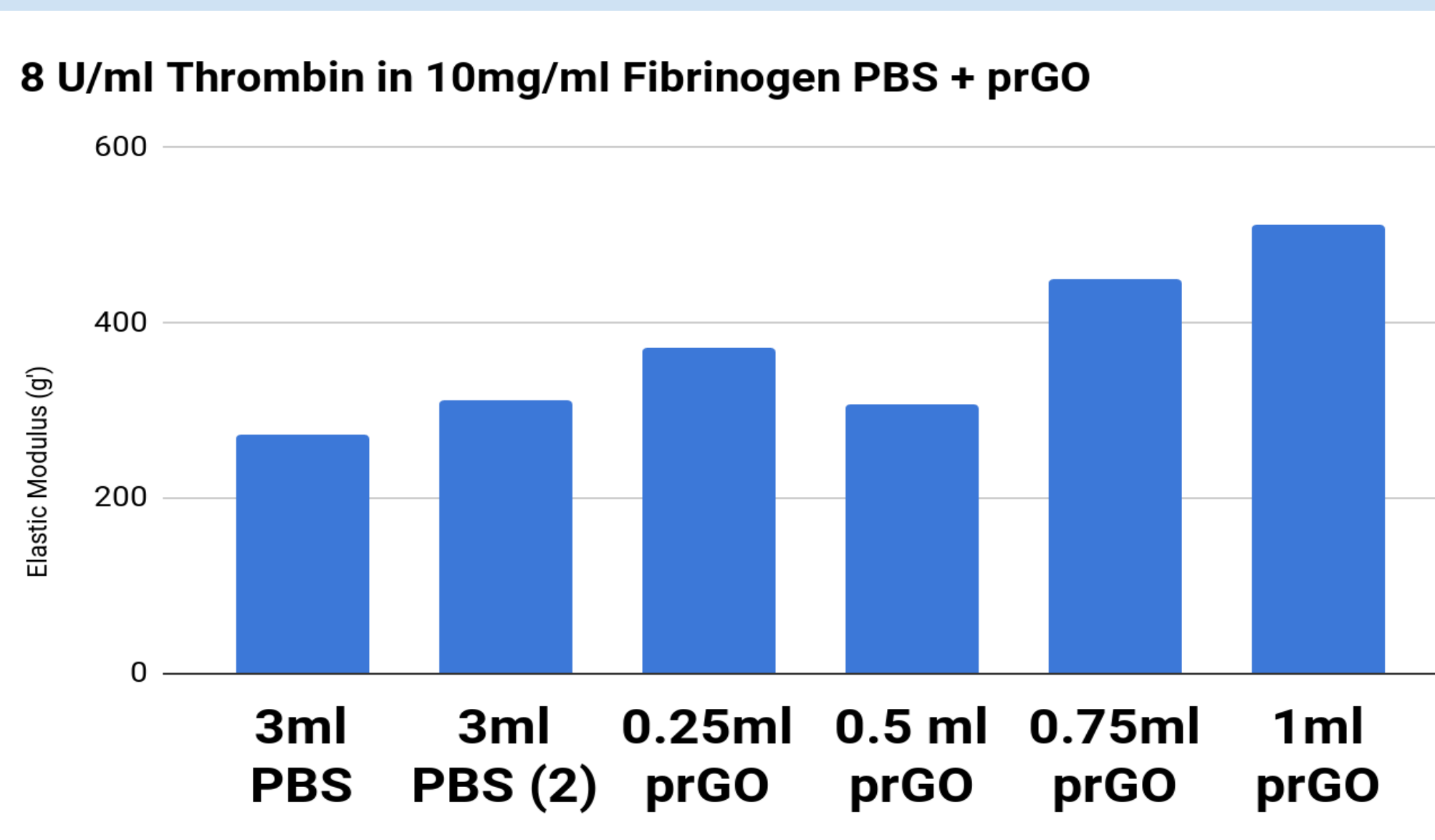


Fig 6. Higher concentrations of pRGO generally had higher elastic moduli, indicating that 1mL was the most effective concentration of pRGO.

Solution	Elastic Modulus
3ml PBS	271.6
3ml PBS (2)	310.6
0.25ml prGO	371.4
0.75ml prGO	449.6
1ml prGO	511.9

Conclusions

- Partially Reduced Graphene Oxide enhances the activity of thrombin, improving its catalytic ability.
 - Increases clot modulus more than four times that of clotting fibrinogen with thrombin alone.
 - Significantly reduces clotting time as well.

Applications

- Wide-ranging applications in biomedical sciences
- Post-operative procedures
- Car accidents
- Traumatic injury

Future Work

- Test to determine the optimal concentration of pRGO for thrombin.
- Study the mechanism by which GO and pRGO enhance thrombin
- Thrombin relies on mobility. Does pRGO enhance this?
- Test different reductions of GO, using different concentrations of Sodium Borohydride
- What effects will pRGO have on the environment? Its safety/toxicity must be tested both on the environment and on humans.

Bibliography

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