



# Improving the Stability of Chitosan-Gelatin Based Injectable Hydrogel

## for Cardiac Regeneration Therapy

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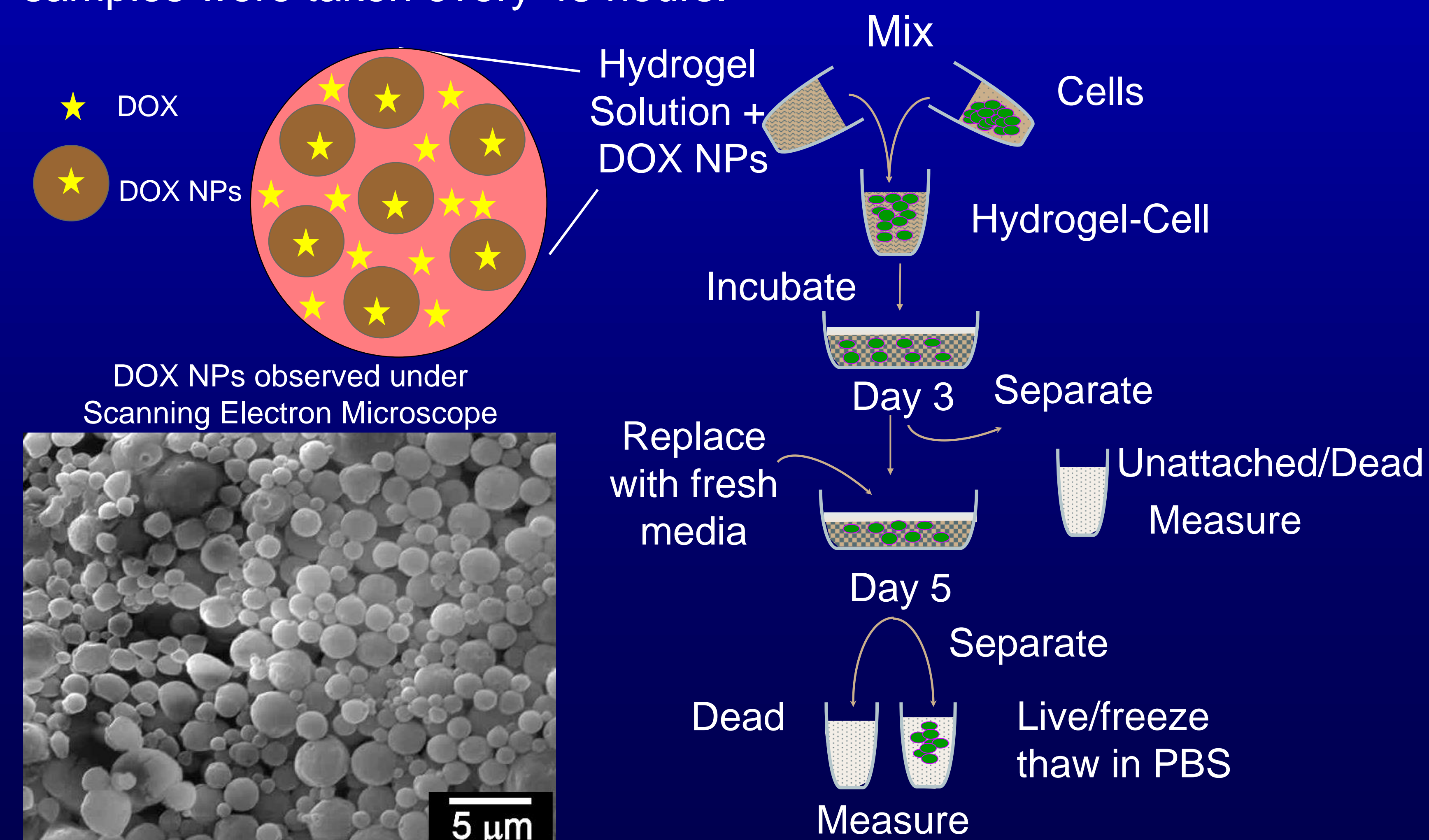
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### INTRODUCTION

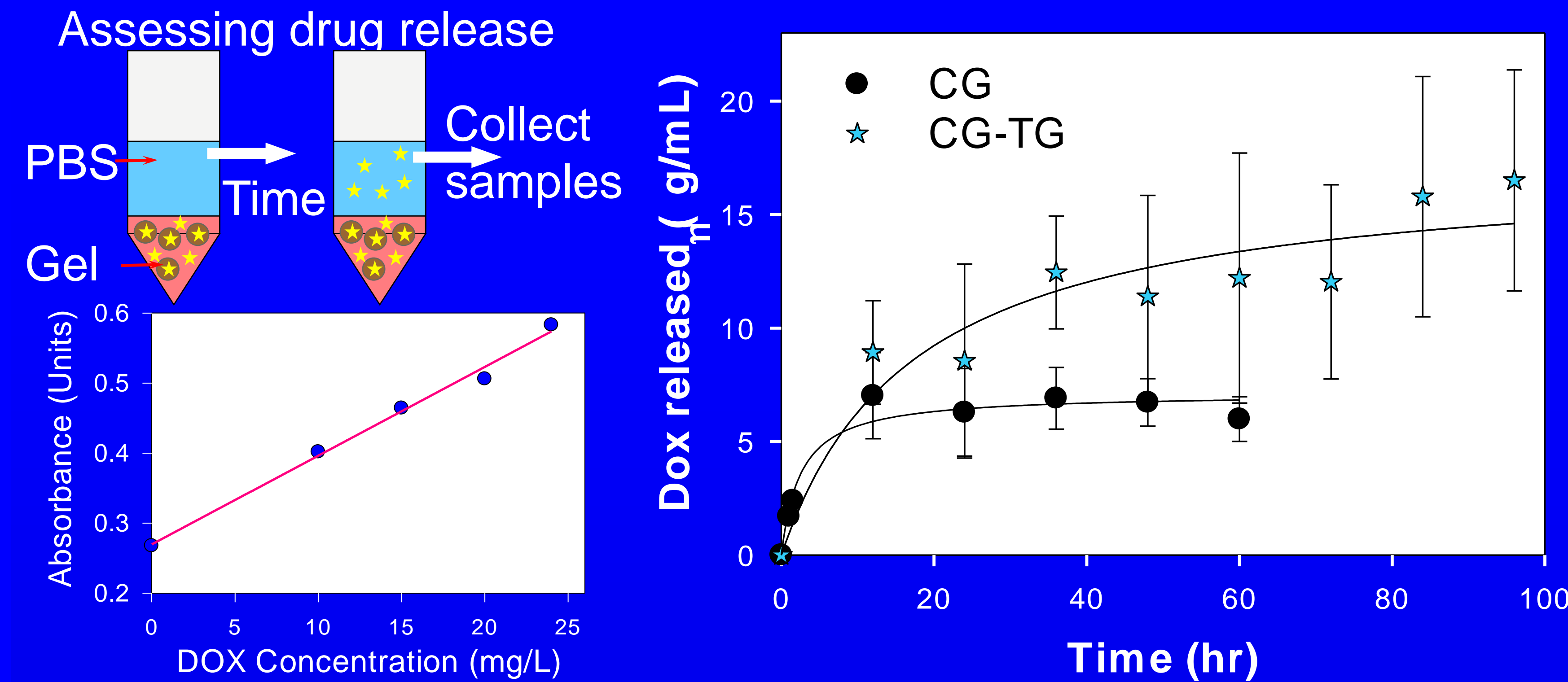
- Delivering cells using hydrogels to an injured area has been attractive option for in situ tissue regeneration.
- Clinical trials show significant attrition of injected human stem cells due to the poor stability of the hydrogel and lack of nutrients.
- To increase the stability of the resulting hydrogel and improve cell retention, Chitosan-gelatin (CG) with growth media was cross-linked with transglutaminase (TG).
- Cells secreted increased amounts of matrix metalloproteases (MMP-2/MMP-9) upon sensation of gelatin.
- Doxycycline hyclate (DOX), an antibiotic and inhibitor of MMP-2/MMP-9, was encapsulated in poly (lactic-co-glycolic acid) (PLGA) nanoparticles (NP) and embedded in the hydrogel.
- Release kinetics were determined and samples were evaluated for protein content.
- Cell viability was also determined by prestaining them with a fluorescent non-toxic dye.

### Cell Culturing with the hydrogel

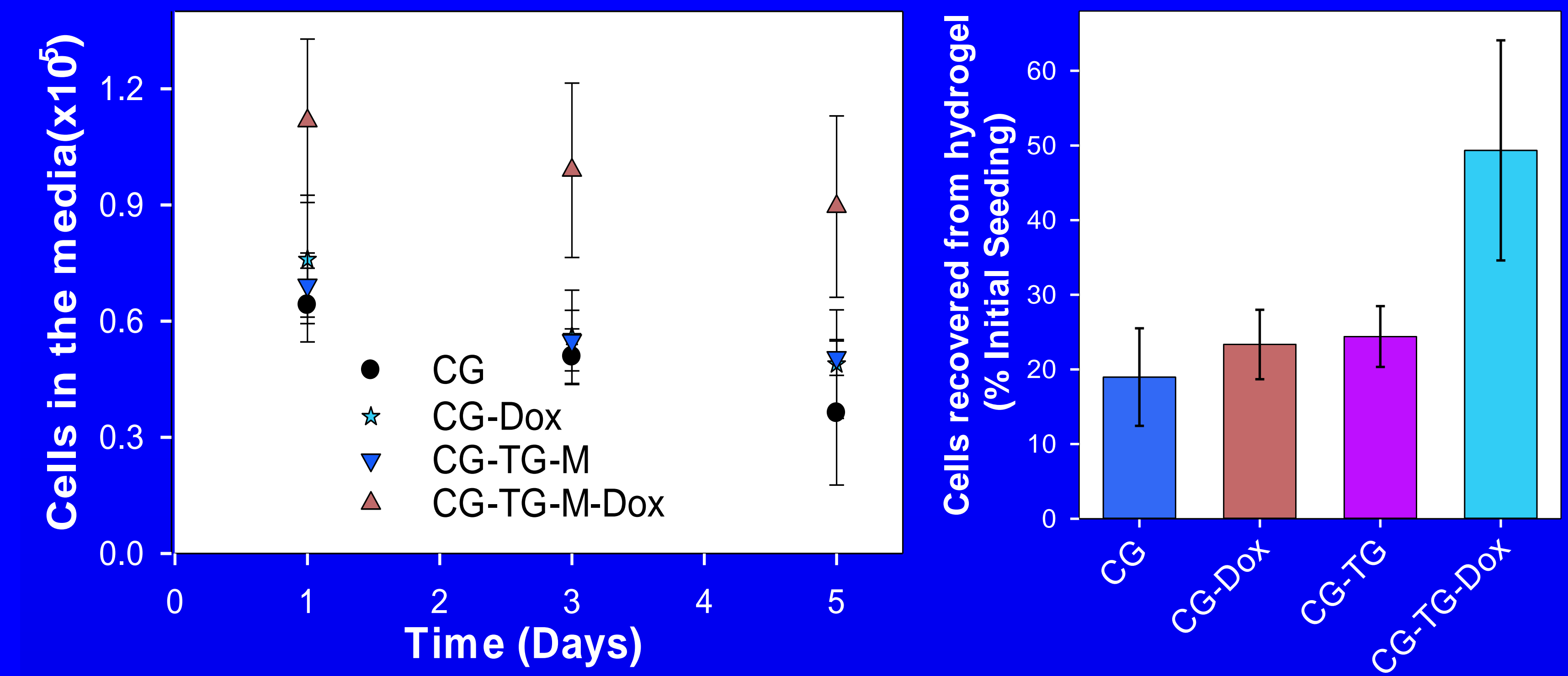
DOX NPs were prepared and added to 2% Chitosan Gelatin hydrogel along with cells. The resulting solution was incubated and samples were taken every 48 hours.



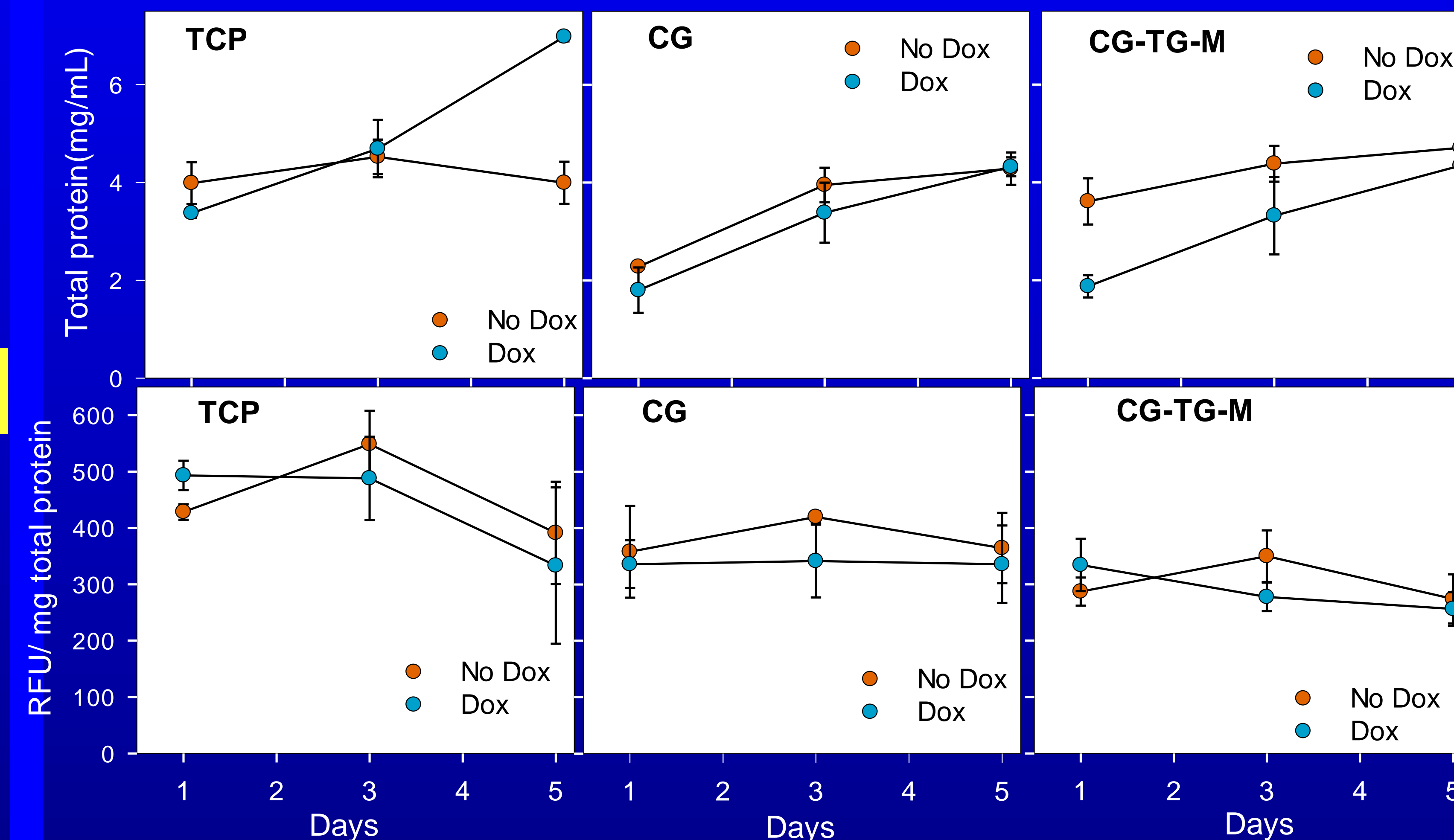
### Improved DOX Release Time with TG



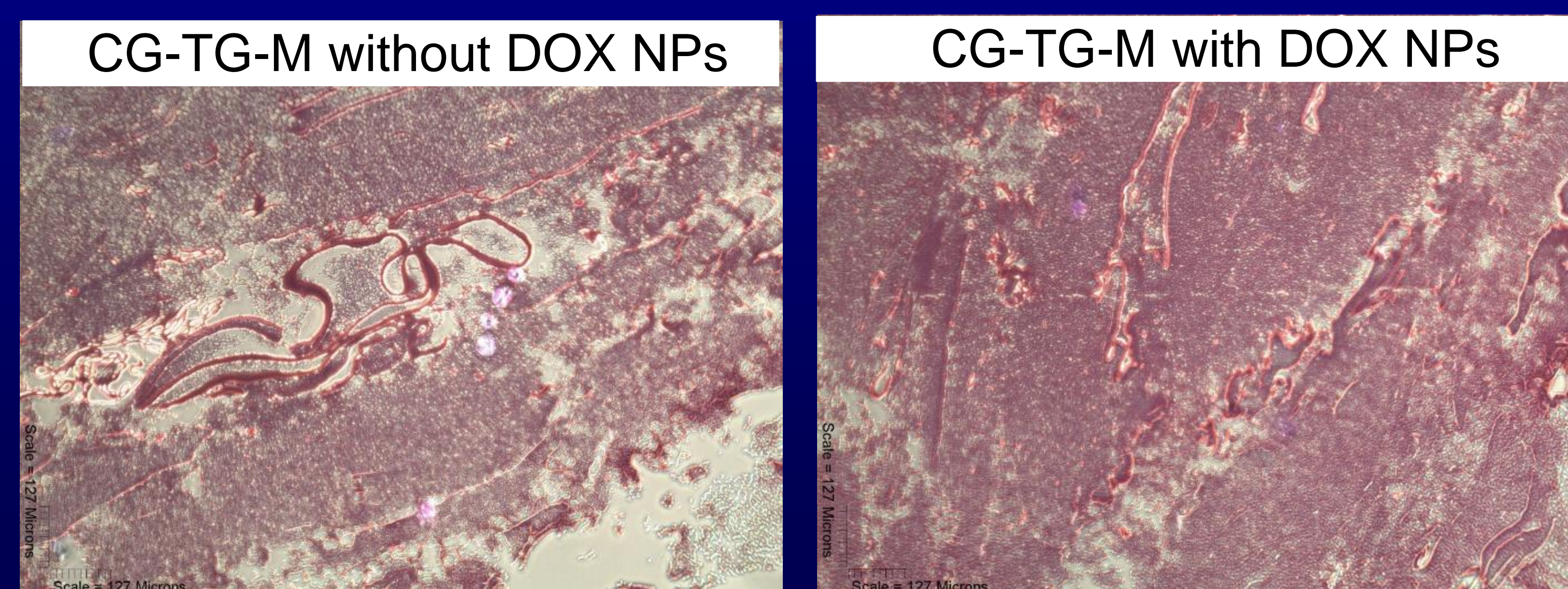
### Improved Cell Retention With DOX and TG



### Prolonged Inhibition of Proteases by DOX



### Histology of Hydrogels



### Societal Impact

With this research, this injectable hydrogel can be used to deliver human adult stem cells along with signaling molecules to stimulate stem cell differentiation in the cardiac region.

### Summary

- DOX NPs were successfully formed and the particle size analysis showed 200-500 nm distribution, which was confirmed by SEM.
- Cross-linking of CG with TG showed an increased, sustained delivery of DOX as a function of time.
- Cell cultures showed no toxicity from DOX at the proposed concentration.
- Cell retention increased by over 250% with the combination of CG-TG-DOX. All cells were accounted for.
- Embedded DOX NPs decreased cell secretion of MMP-2/MMP-9.

### Work in Progress

- Add signaling molecule involved in direct stem cell differentiation to injectable hydrogel.
- Testing long-term viability of human adipocyte stem cells.

### ACKNOWLEDGEMENTS

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