# Injectable Macroporous Hydrogel for Wound Healing and *In Situ* Cell Encapsulation

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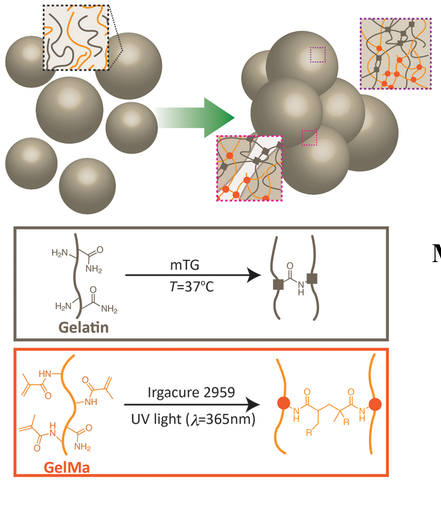
## Abstract

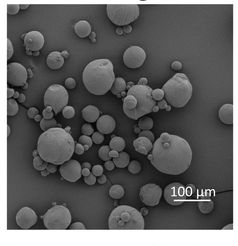
Injectable hydrogels are of high interest for use in regenerative medicine due to their high water content and ability to conform to the shape of surrounding tissue. This platform has previously demonstrated potential as a temporary scaffold for tissue regeneration, or delivery vehicle for cells, growth factors, or drugs. However, most injectable microgel hydrogel systems lack a macroporous structure, preventing host cell migration into the hydrogel interior, and preventing proper spreading and proliferation of encapsulated cells. Herein a novel injectable macroporous hydrogel assembled from gelatin/gelatin methacryloyl (gelMA) composite microgels is described. Multiple crosslinking mechanisms were utilized to enable fast curing, and allowed for the optimization of photoinitiator (PI) concentration to minimize cell death upon UV irradiation. The result is a biodegradable hydrogel system that exhibited rapid gelation, optimized conditions for encapsulated cells, and capability for tissue adhesion, making it appropriate for use in tissue engineering applications.

## Gelatin/GelMA Composite Microgels

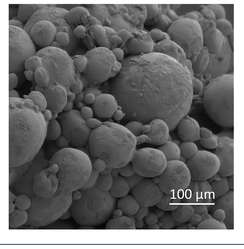
Previously, we reported an injectable macroporous hydrogel by crosslinking gelatin microgels by microbial transglutaminase (mTG) for use in wound healing. The method used to create this hydrogel was simple, requiring no chemical modifications to the starting reagents, cost-effective, and highly bio-functional, allowing for migration of cells into the hydrogel, and providing for optimal cell proliferation. However, despite displaying many advantageous qualities, this technology required a lengthy curing time (~ 60 minutes), limiting its usefulness *in vivo*. Using a similar methodology, a composite gelatin/gelatin methacryloyl (GelMA)-based injectable macroporous hydrogel that cures quickly, is minimally cytotoxic and stably adheres to the applied tissue was developed. This fast-curing hydrogel utilizes dual crosslinking; enzymatic crosslinking of gelatin by mTG, and photocrosslinking of GelMA by ultraviolet (UV) irradiation. Photocrosslinking achieves the initial rapid curing of microgels (< 2.5 minutes) to form a bulk macroporous hydrogel, while mTG strengthens the hydrogel by forming additional covalent crosslinks and allows the resulting hydrogel to adhere to the applied tissue.



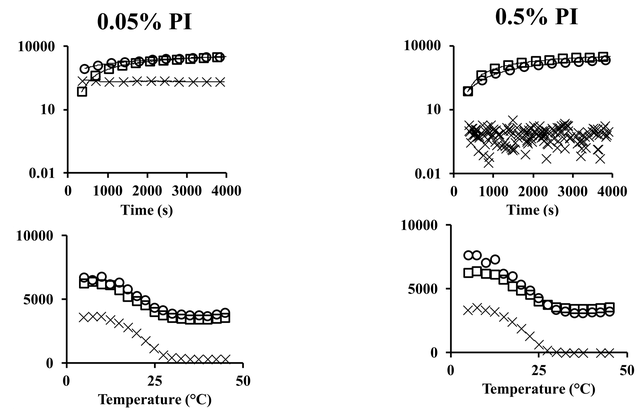

**Microgel**




**Macroporous Hydrogel**



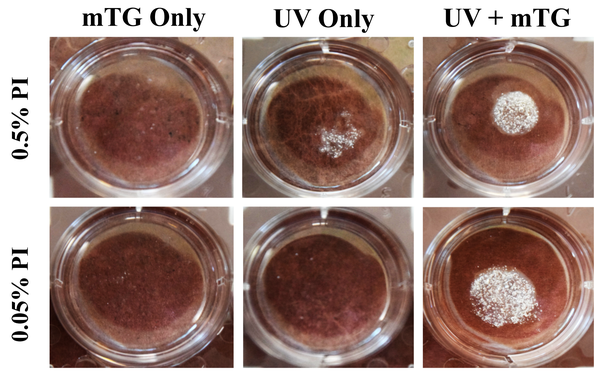

## Rheological Characterization




**Conclusion:** Use of both UV and mTG crosslinking allow for more rapid gelation

## Rapid Curing of Composite Microgels

**Experiment:** Stable gelation was examined by allowing microgel suspensions to gel for 2.5 minutes under various conditions, and then submerged in a warm water bath (45 °C) to assess the formation of chemical crosslinks. Images were taken in a 12 well plate.

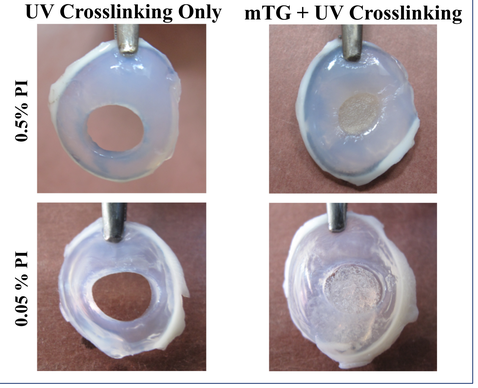



**Conclusion:** Multiple crosslinking methods allow for minimization of photoinitiator concentration

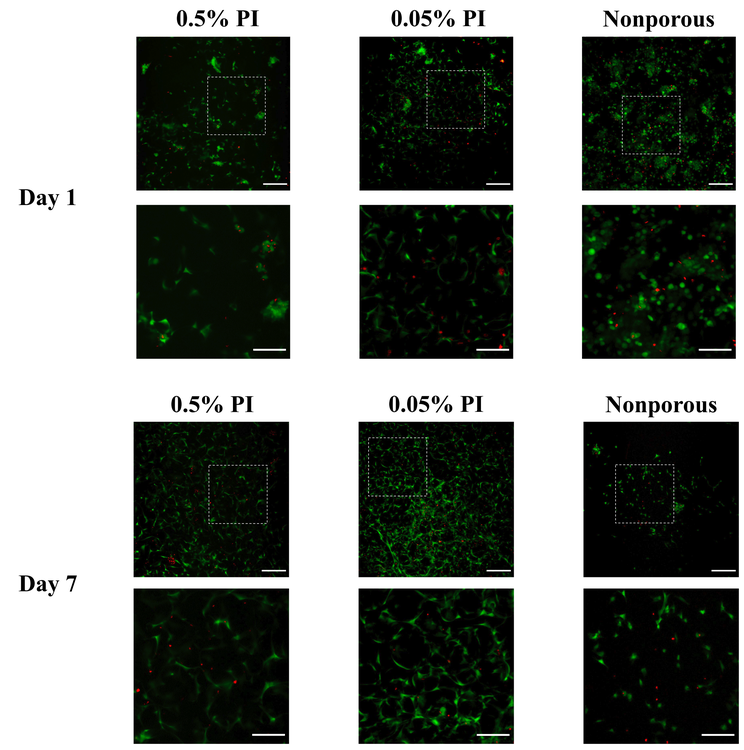
## Tissue Adhesion

**Experiment:** Microgel suspensions were crosslinked in cavities made in porcine corneas by biopsy punches for 2.5 minutes. Corneas were then submerged in 45 °C to assess adhesion to corneal tissue.

**Conclusion:** mTG, known widely as 'meat glue', was able to stable adhere rapidly formed hydrogel to corneal tissue.



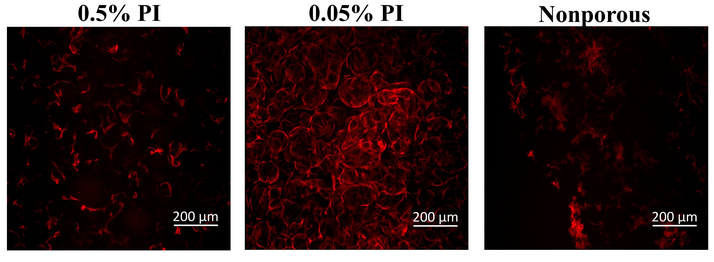

## Live/Dead Assay on Encapsulated Cells: Z Projection




Green: Living Cells

Red: Dead Cells

## Day 7 Actin Cytoskeleton: Z Projection




Red: Actin Cytoskeleton

## Conclusions

- Drastically decreased gelation time in comparison to gelatin macroporous hydrogel, enhancing clinical relevance
- Dual crosslinking due to action of mTG and UV photocrosslinking allow for minimization of photoinitiator concentration, lowering cytotoxic effects from UV exposure.
- Use of mTG allows for scaffold to adhere to surrounding tissue.
- The presence of macropores allow for optimal growth and expansion of encapsulated cells, and infiltration of host tissue cells.

## Acknowledgements

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