Temporal Zinc Release from Hydrogels: Effects on Mechanics, Metabolic Activity, and Gene Expression

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Introduction

- Localized delivery of zinc at surgical fracture fixation sites has promising therapeutic potential:
  - Zinc delivery via oral ingestion, injection, and nanoparticle carriers were shown to enhance osteoblast differentiation and inhibit osteoclast differentiation [1]
  - Zinc upregulates ALP, COL1A, and RUNX2 via cAMP-PKA-CREB signaling pathway [2]
- Gelatin methacryloyl (GelMA) is a photocrosslinkable hydrogel that is biocompatible, biodegradable, and has tunable mechanical properties [1]

Knowledge Gap: Is GelMA an effective vehicle to provide sustained release of zinc at a fracture site?

Objective: Determine changes in degradation kinetics and osteogenic effects caused by differences in zinc dosage and method of application (direct seeding v. transwell co-culture)

Hypotheses:

- Addition of $ZnCl_2$ to GelMA will not significantly change its degradation kinetics or mechanical properties
- Increased dosage of $ZnCl_2$ improves metabolic activity

Fabrication of GelMA

5% (w/v) GelMA

- Gelatin methacryloyl powder + DPBS in 5% w/v + 0.05% (w/v) Irgacure 2959
- Add 100μM $ZnCl_2$, 0.1M $ZnCl_2$, and 1M $ZnCl_2$ for zinc-doped GelMA
- Cure under 365nm UV light at 65 mW/cm^2 for 5 minutes
- Create cylindrical samples (3mm thickness, 5mm dia.) via biopsy-punch from cured hydrogel

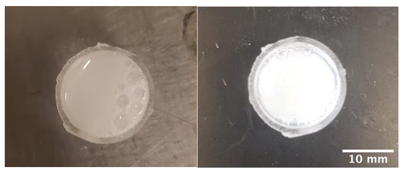


Figure 1. Left: 5% GelMA + 0.1M $ZnCl_2$. Right: 5% GelMA + 1M $ZnCl_2$.

Mechanical Testing/Degradation

Unconfined Compression

- Creep test at 4.9 N for 300 seconds
- Stress relaxation following creep test for 1000 seconds
- Cyclic loading to 1% of sample thickness at 0.5 Hz for 10 cycles
- Equilibrium modulus calculated from creep strain and stress from stress relaxation

Degradation

- Samples were incubated in DPBS for 24 hours
- Initial dry weight was taken after incubation
- Weight was measured everyday until day 7 and every 2 days until complete degradation
- $\%\ \text{mass remaining} = \frac{W_{initial} - W_{dry}}{W_{initial}} \times 100\%$

In Vitro Studies

Approach 1: Direct Seeding

- 100,000 bovine mesenchymal stem cells (bMSCs) were seeded directly on top of samples (**Fig. 2A**)
- Tested 5% GelMA and 5% GelMA + 100μM $ZnCl_2$

Approach 2: Transwell Co-culture

- 100,000 bMSCs were seeded on the bottom chambers of 12-well plates
- 5% GelMA, 5% GelMA + 0.1M $ZnCl_2$, 5% GelMA + 1M $ZnCl_2$ samples were placed in the transwell insert (**Fig. 2B**)

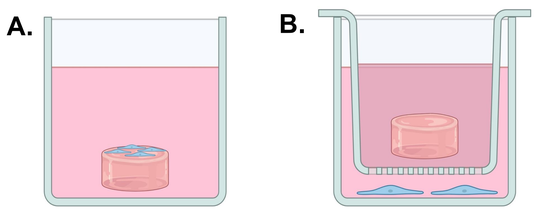


Figure 2. A) Schematic of approach 1: direct seeding of bMSCs on GelMA sample. B) Schematic of approach 2: hydrogel in transwell insert and bMSCs seeded on well bottom.

Metabolic Activity

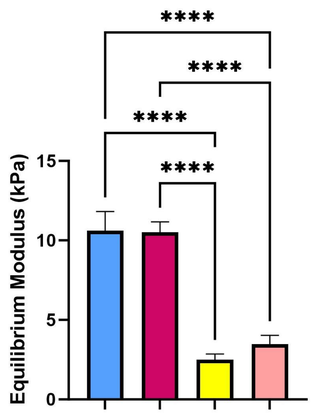
- bMSCs were cultured in DMEM (+10% FBS, 1% Penicillin-streptomycin)
- Alamar blue assays were conducted on day 1, 3, 7, 14, 21, and 28
- Measured fluorescence at 560-590 nm wavelength

RT-qPCR

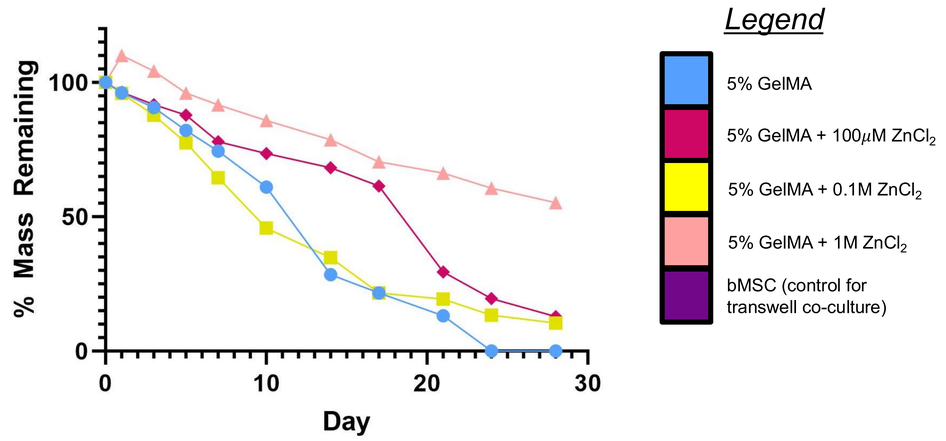
- RT-qPCR conducted on day 7 and 28
- Measure relative gene expression of ALP and COL1a, with GAPDH as housekeeping gene
- No significant results (not shown)

Results

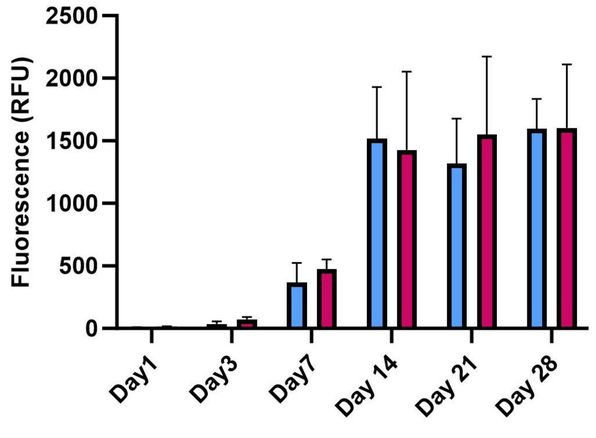
A. Mechanical Testing



B. Degradation Kinetics



C. Metabolic Activity Direct Seeding



D. Metabolic Activity Transwell

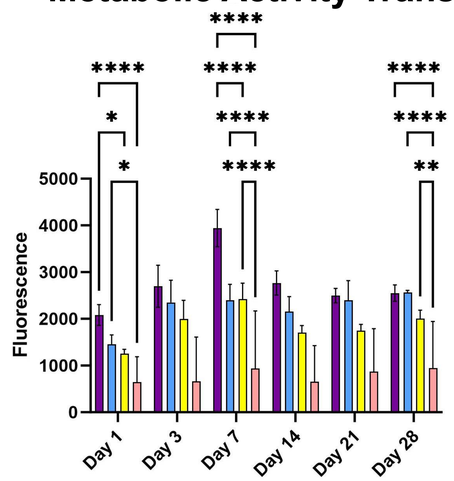


Figure 3. A) Equilibrium moduli was unaffected by 100μM concentrations of $ZnCl_2$ but significant losses were observed at higher concentrations. B) The 28-day degradation study that showed that inclusion of $ZnCl_2$ decelerated degradation kinetics. C) Metabolic activity for 5% GelMA and 5% GelMA + 100μM $ZnCl_2$, where bMSCs were directly seeded onto the GelMA samples. There were no significant differences between groups. D) Metabolic activity for bMSC control, 5% GelMA, 5% GelMA + 0.1M $ZnCl_2$ and 5% GelMA + 1M $ZnCl_2$, where GelMA samples co-cultured with bMSCs and did not directly contact cells. *Note:* Significance only shown for Day 1,3,28 for for panel D. *p<0.05, ***p<0.001, ****p<0.0001.

Conclusions

- Addition of $ZnCl_2$ to GelMA with concentration > 100μM $ZnCl_2$ significantly changed mechanical properties.
- Presence of $ZnCl_2$ led to substantial deceleration of degradation
- Excess $ZnCl_2$ (1M) inhibited the metabolic activity of bMSCs
- Study did not elucidate advantages of using a transwell co-culture approach in comparison to direct seeding

Future Directions

- This work was preliminary in nature
- Conduct RT-qPCR for transwell co-culture approach
- Explore 3D printed zinc-doped GelMAs
- Define uses for zinc-doped GelMAs in combination with other biomaterials through in vitro and vivo studies

References & Acknowledgements

References: [1] M. Sun, et al., Polymers, 2018. [2] K.H. Park, et al., Stem Cells and Development, 2018.

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