

Supporting Information

On-demand Release of a Selective MMP-13 Blocker from an Enzyme-Responsive Injectable Hydrogel Protects Cartilage from Degenerative Progression in Osteoarthritis

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1. Cytocompatibility studies of BI-4394

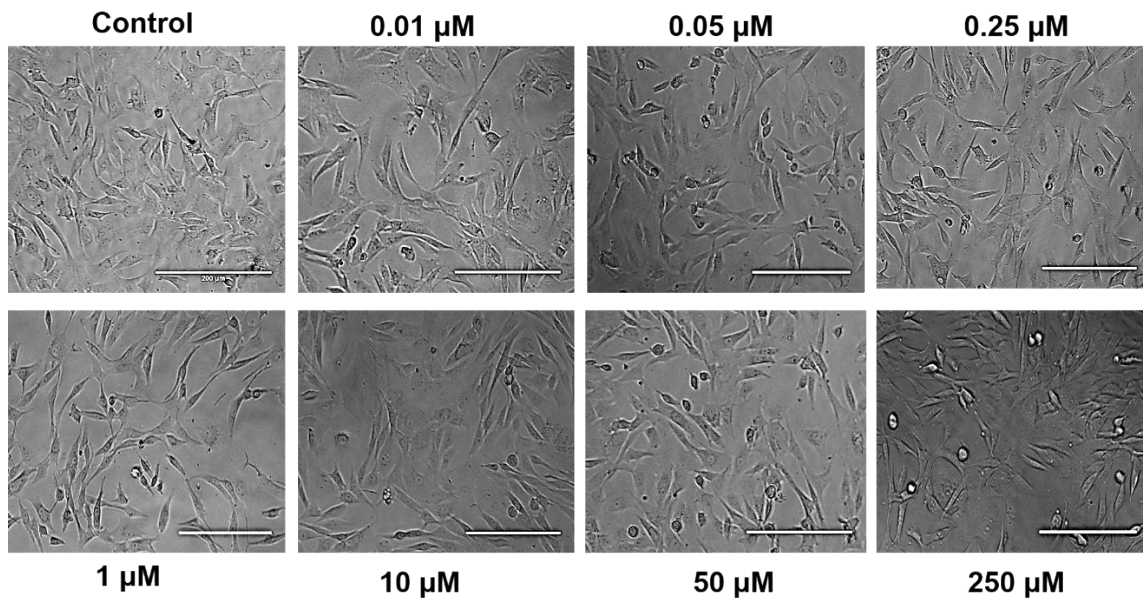


Fig S1: Representative images of human articular chondrocytes treated with BI-4394. Scale bar represents 200 μ m.

2. Gene regulation with BI-4394

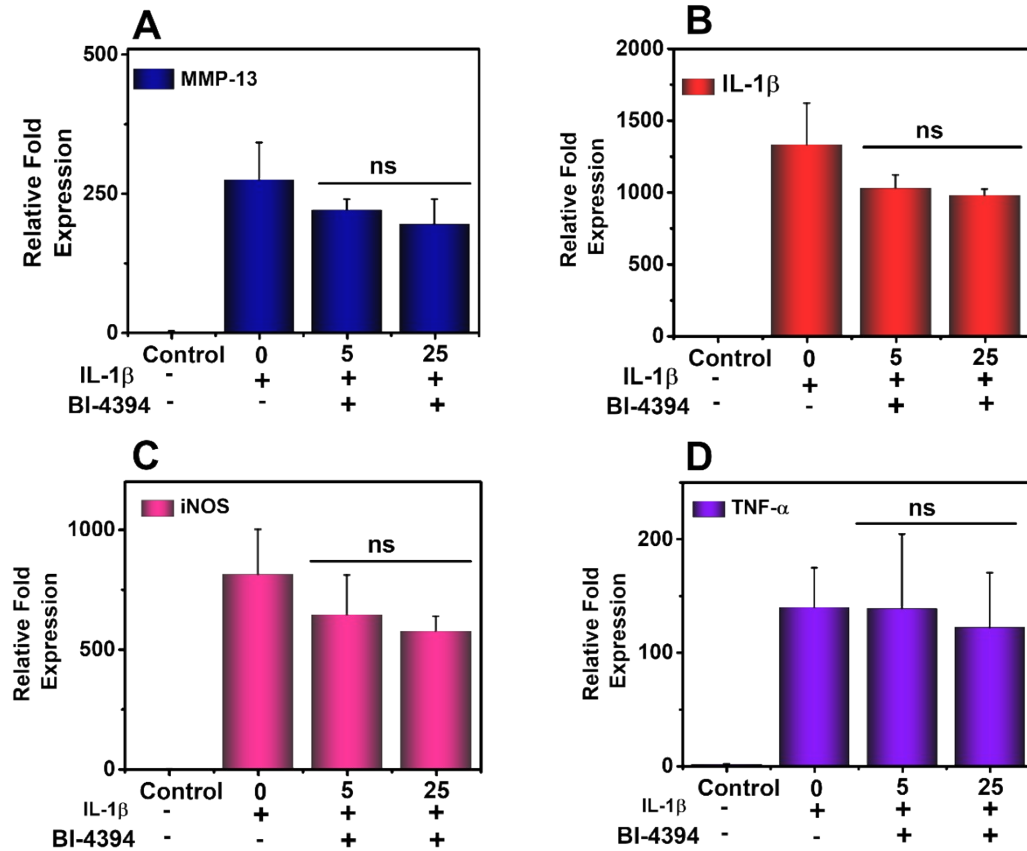


Fig S2: Gene expression in chondrocytes treated with IL-1 β and BI-4394 respectively. (A) MMP-13; (B) IL-1 β ; (C) iNOS; (D) TNF- α . “ns” denotes no significant difference in expression in comparison to IL-1 β treated group.

3. Characterization of BI-4394 loaded hydrogels

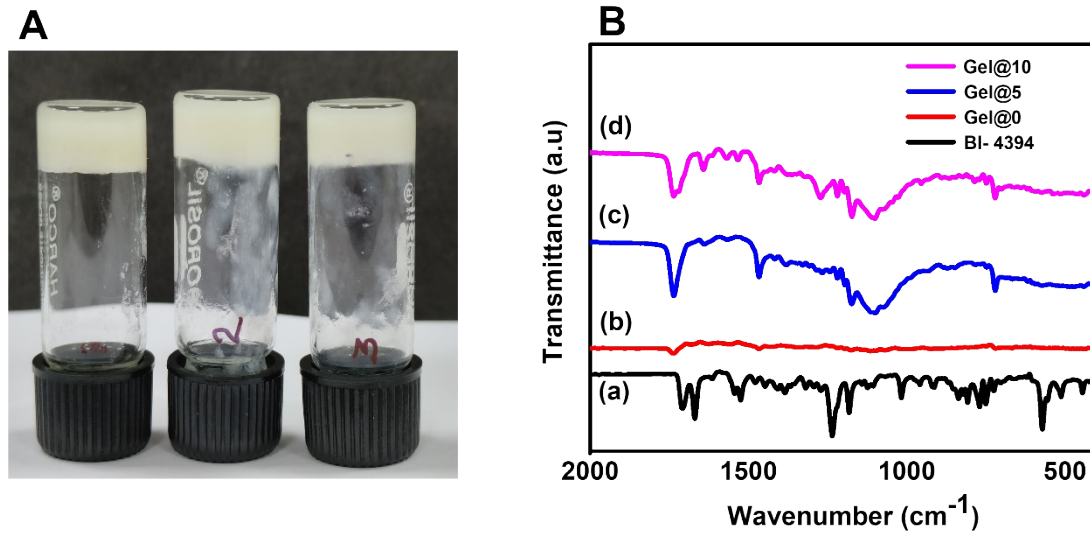


Fig S3: Characterization of BI-4394 loaded hydrogel. (A) Representative images of hydrogels. Blank hydrogel (left); (2) 5 mg/mL loaded BI-4394 (middle); (3) 10 mg/mL loaded BI-4394 (right); (B) FTIR.

4. Cytocompatibility studies of hydrogel

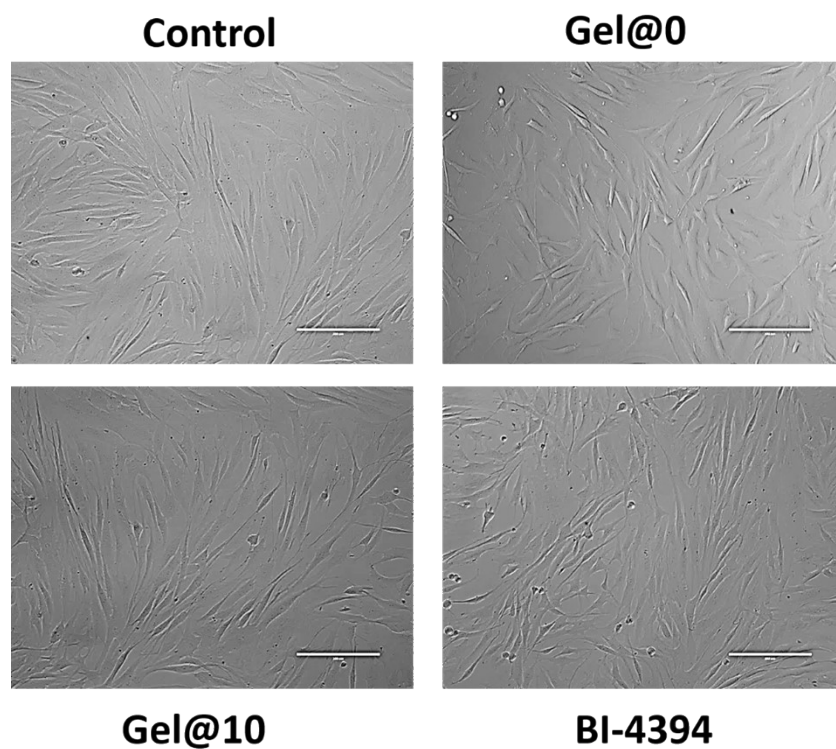


Fig S4: Representative images of human articular chondrocytes treated with BI-4394 loaded hydrogels. Scale bar represents 200 μm .

5. Rheology of the hydrogels

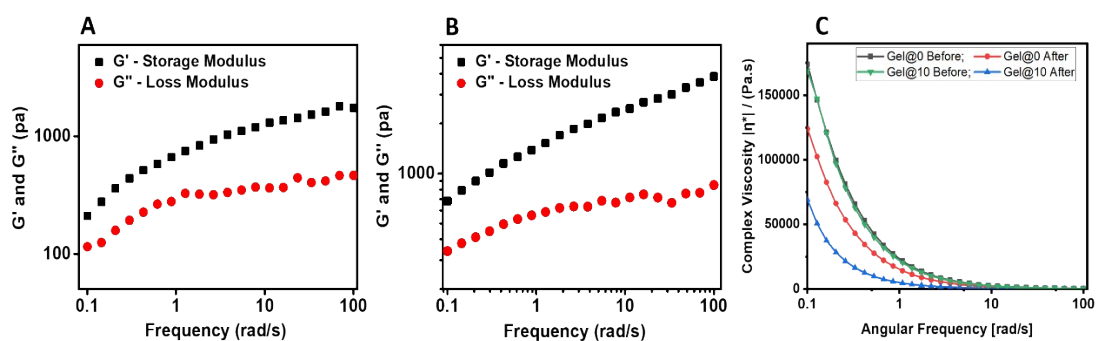


Fig S5: Frequency sweep (A) Blank hydrogel (Gel@0); (B) BI-4394 loaded hydrogel (Gel@10); (C) Gel viscosity of the Gel@0 and Gel@10 before and after injection with a shear rate ranging from 0.1 to 100 rad/s at 25 $^{\circ}\text{C}$.

6 *In-vivo safety evaluation of hydrogels*

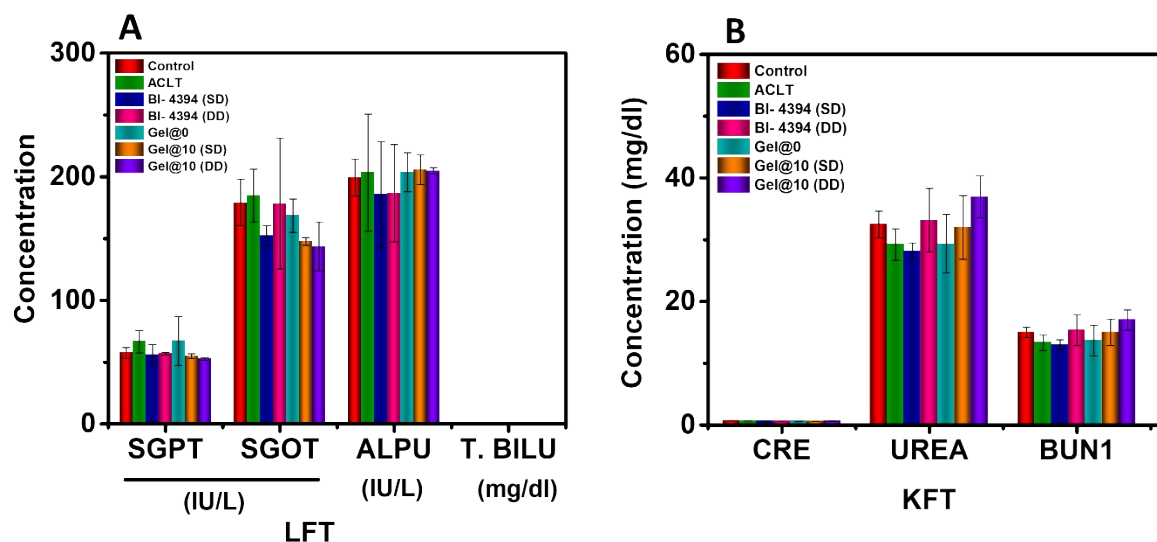


Fig S6: Estimation of markers for (A) Liver function test (LFT); (B) Kidney function test (KFT).

Table 1: Table for OARSI scoring guidelines.

OARSI Guidelines Osteoarthritis Research Society International.		
A	Structure	Score
1	Normal score.	0
2	Slight surface irregularities.	1
3	Moderate surface irregularities.	2
4	Severe surface irregularities.	3
5	Clefts/fissures into transitional zone (one-third depth).	4
6	Clefts/fissures into radial zone (two-thirds depth).	5
7	Clefts/fissures into calcified zone (full depth).	6
8	Fibrillation and/or erosion to transitional zone (one-third depth).	7
9	Fibrillation and/or erosion to radial zone (two-thirds depth).	8
10	Fibrillation and/or erosion to calcified zone (full depth).	9
11	Fibrillation and/or erosion to subchondral bone.	10
B	Cellularity	

1	Normal score.	0
2	Increase or slight decrease.	1
3	Moderate decrease.	2
4	Severe decrease.	3
5	No cells present.	4
C	Chondrocytes Cloning	
1	Normal score.	0
2	Several doublets.	1
3	Many doublets.	2
4	Doublets and triplets.	3
5	Multiple cell nests.	4

7 *In-vivo* OARSI Scoring

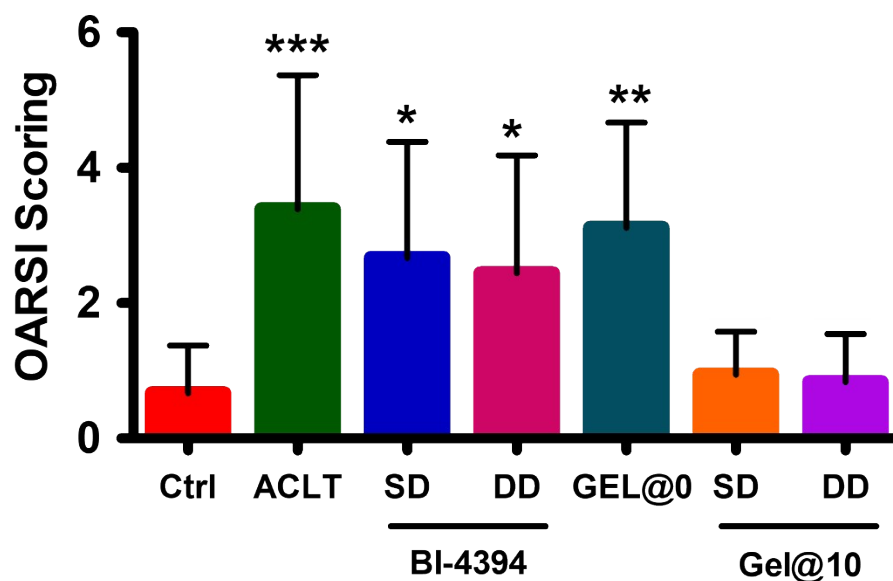


Fig S7: OARSI scoring of microscopic evaluation of histological sections. One-way ANOVA, Dunnett's multiple comparisons test. * represents the level of significance in comparison to control group. (** $P \leq 0.0001$, ** $P \leq 0.01$ and * $P \leq 0.05$).

8 *In-vivo* evaluation of MMP-13

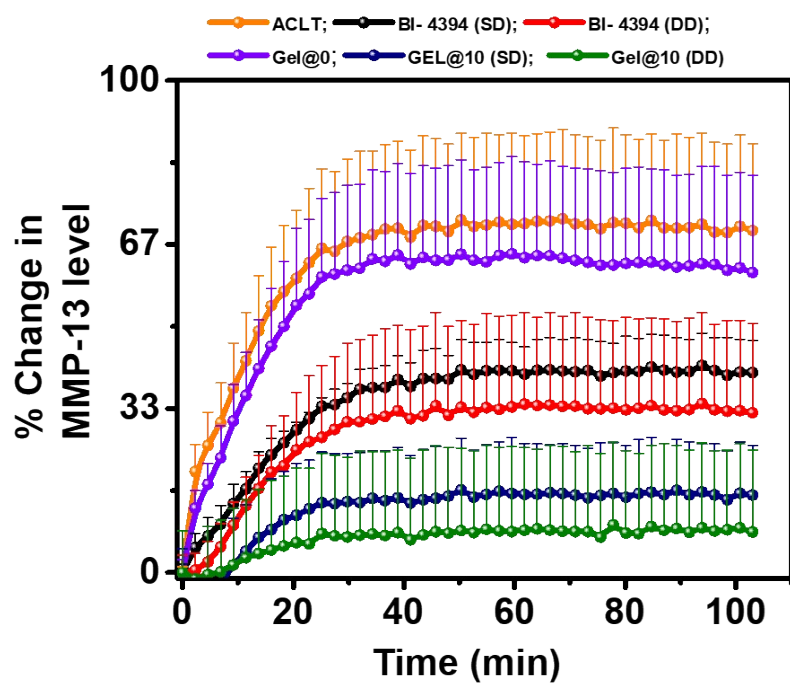


Fig S8. MMP-13 levels in the respective rat serum.