Electronic Supplementary Material (ESI) for Polymer Chemistry

Supporting Information

Enzyme-Mediated Fast Injectable Hydrogels Based on Chitosan-Glycolic acid/Tyrosine: Preparation, Characterization, and Chondrocyte Culture

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Experimental Section

Cell proliferation assay

The analysis of the chondrocyte proliferation was performed using AlamarBlue assay (10×, Invitrogen). In brief, at day 1, 7 and 14, the medium was removed and the cells were washed twice with fresh D-Hanks buffer and incubated with freshly made 1×diluted AlamarBlue solution (in RPMI 1640). After 4 h, 200 μl of the medium solution from each well was transferred to a 96-well plate. The optical density (OD) was measured using a plate reader (Thermo Fisher Scientific) at 570 nm.

Cell morphology characterization

The morphology of chondrocytes in tyrosinase-crosslinked CH-GA/Tyr hydrogel was studied using a scanning electrn microscopy (SEM). After cultured for 14 days *in vitro*, the cell/gel construct was fixed with formalin and sequential dehydrated. The sample was then dried at a critical point, coated with gold and analyzed with SEM.

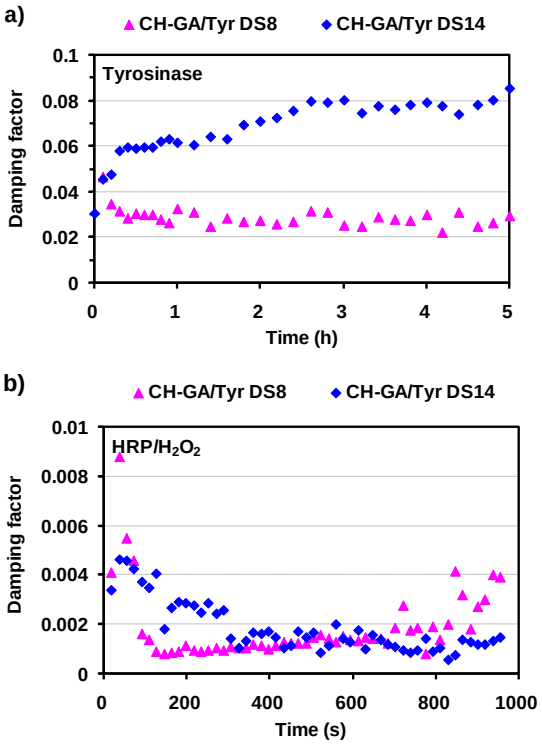

Figure S1. Damping factors of injectable 2 wt% CH-GA/Tyr hydrogels crosslinked by (a) tyrosinase or (b) HRP/H_2O_2.

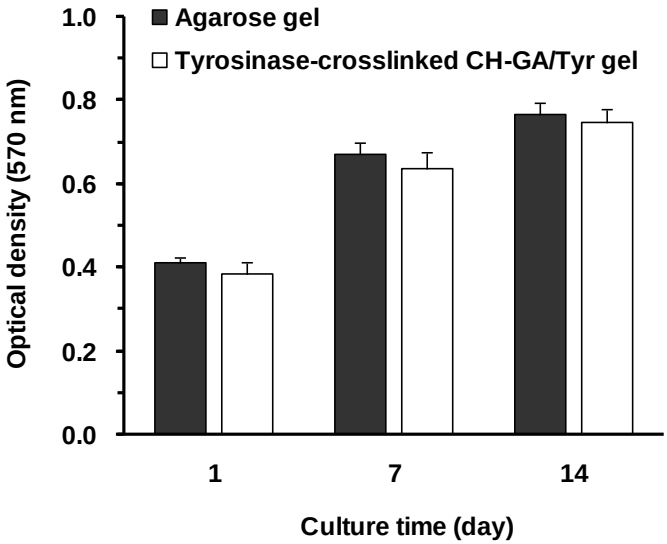

Figure S2. AlamarBlue assay showing cell proliferation profile of the chondrocytes as a function of culture time after the cells were encapsulated in the 4 wt% tyrosinase-crosslinked CH-GA/Tyr DS14 hydrogel. Agarose hydrogel was also shown as a positive control.

Figure S3. SEM image of chondrocytes encapsulated in the 4 wt% tyrosinase-crosslinked CH-GA/Tyr DS14 hydrogel at day 14. Scale bar: 10 μm.