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Supporting Information

Preparation of Mussel-Inspired Injectable Hydrogels Based on Dually Functionalized Alginate with Improved Adhesive, Self-Healing, and Mechanical Properties

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Synthesis of PLGA-ADH and ALG-CHO

For the preparation of hydrazide-modified PLGA (PLGA-ADH), PLGA was dissolved in distilled water and then reacted with ADH in the presence of EDC and NHS.[1] The -COOH of PLGA/ADH/EDC/NHS molar ratio was set at 1:3:2:1. The pH of the reaction mixture was adjusted to 5.5 by the addition of 0.1 M NaOH or HCl solution. The reaction was allowed to continue at room temperature for 24 h. Then, the solution was exhaustively dialyzed against deionized water for 3d followed by lyophilization.

ALG was modified to aldehyde form (ALG-CHO) using $NaIO_4$ as oxidizing agent, according to our previously reported procedure.[2] An aqueous solution of ALG (5 wt%) was oxidized in $NaIO_4$ solution. The molar ratio of $NaIO_4$ to saccharide repeating units of ALG was set at 0.2. The oxidation reaction proceeded at room temperature in the dark for 3 h. The reaction was stopped by adding excess amount of ethylene glycol in a molar ratio of $-$OH to $NaIO_4$ of at least 3. Then the solution was dialyzed immediately against distilled water for 3 days. The final purified oxidized ALG product was obtained by freeze-drying.

The ADH modification of PLGA-ADH determined from the relative peak area of ^{1}H NMR spectra and oxidation degree of ALG-CHO evaluated by hydroxylamine hydrochloride titration potentiometric were 41% and 28%, respectively.[1]

Swelling and degradation of hydrogels

Swelling and degradability of the dried hydrogels in PBS buffer solution was determined gravimetrically, according to our previously reported procedure.[1]

With the increase of Catechol grafting ratio from 9.4% to 26.1%, the swelling ratio of PLGA/ALG-CHO-catechol hydrogel increased from 19.5±2.3 to 29.5±2.4 (Fig. S1a), which was corresponding to the hydrophilicity of ALG-CHO-Catechol with different catechol grafting ratios (Fig. 2e). When the solid content increased from 5 wt% to 7wt%, the swelling ratio of PLGA/ALG-CHO-catechol hydrogel and oxidized ALG-CHO-catechol hydrogel decreased from 29.5±2.4 to 17.1±1.8 and 25.0±2.3 to 10.1±1.3, respectively (Fig. S1b), which could be ascribed to the increasing

amount of reactive groups and cross-linking density of the network.

Degradability of the hydrogels was shown in Fig. S1c and S1d. For both PLGA/ALG-CHO-catechol hydrogels and oxidized ALG-CHO-catechol hydrogels, rapid degradation was observed within 3 to 7 days, which is presumably due to the gel erosion and removal of the non-cross-linked macromolecular chains. The PLGA/ALG-CHO-catechol hydrogels with solid contents of 5 wt%, 6 wt%, and 7 wt% displayed weight loss of 53.7%, 49.7%, and 42.9% after 6 weeks of incubation. And the oxidized ALG-CHO-catechol hydrogels with solid contents of 5 wt%, 6 wt%, and 7 wt% experienced weight loss of 44.1%, 38.0%, and 40.0% after 4 weeks. Higher solid content led to a higher resistance to biodegradability because of higher cross-linking density of the network and more compact network structure of the hydrogel. Compared with the oxidized ALG-CHO-catechol hydrogels, PLGA/ALG-CHO-catechol hydrogels exhibited higher resistance to biodegradability.

Self-healing ability of hydrogels

Two pieces of PLGA/ALG-CHO-Catechol hydrogels of different colors were immediately combined into a new hydrogel with enough joint strength to support self-weight, as shown in Video S1. Also the evaluation of the self-healing behavior of the PLGA/ALG-CHO hydrogels was shown in Fig. S2.

In vitro cytotoxicity assay and biocompatibility of hydrogels

Adipose-derived stem cells (ADSCs) were isolated from adipose tissue of New Zealand rabbits.[3] The ADSCs were encapsulated in the hydrogels (0.5×10^6 cells/mL) under sterile condition by filtrating the precursor PBS solution (5 wt%) of PLGA-ADH and ALG-CHO-Catechol through a pore size of 0.22 μm, according to the previously reported procedure.[2] After 1d, 4d, and 7d of celluar culture, the cell/hydrogel constructs were stained with fluorescein diacetate/propidium iodide (FDA/PI) using the live-dead assay kit (Invitrogen), according to the manufacturers' instructions and visualized using fluorescence microscopy (Leica). The morphology of the ADSCs in the hydrogels was observed via a desktop SEM (Phenom G2 pro, Holland).

As shown in Fig. S3a-S3c, the majority of cells encapsulated in hydrogels were

shown to survive after 1d, 4d, and 7d. The morphology of ADSCs in PLGA/ALG-CHO-Catechol hydrogel was also observed using SEM. As shown in Fig. S3d and Fig. S3e, we could find that the round shaped ADSCs were uniformly distributed. At 7 days of culture, some extracellular matrix deposition was found on the surface of the cell–hydrogel complex (Fig. S3f), indicating cytocompatibility of the hydrogels.

Wet adhesion behavior of hydrogels

After the porcine bone filled with PLGA/ALG-CHO-catechol hydrogel was soaked in 0.01 M PBS at 37℃ for 24 h and flushed under faucet for 5 min, the hydrogel still maintained its original shape and no sign of detachment of the hydrogel from the bone defect was observed, as demonstrated in Video S2.

Reference

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2 S. Yan, T. Wang, L. Feng, J. Zhu, K. Zhang, X. Chen, L. Cui and J. Yin, *Biomacromolecules,* 2014, **15**, 4495-4508.

3 K. Zhang, S. Yan, G. Li, L. Cui and J. Yin, *Biomaterials,* 2015, **71**, 24-34.

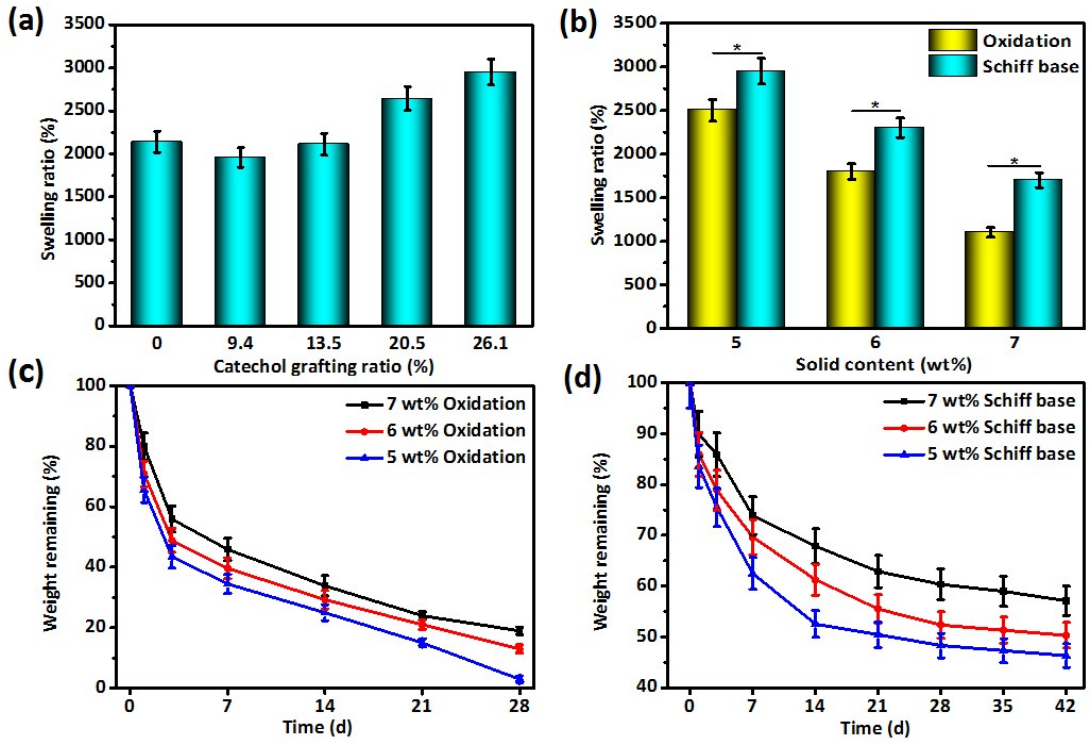

Fig. S1 Equilibrium swelling and degradation of the hydrogels. Equilibrium swelling of the PLGA/ALG-CHO-Catechol hydrogels as a function of (a) solid content and (b) catechol grafting ratio (*, $p \leq 0.05$, n = 3). Degradation of the hydrogels with various solid contents: (c) oxidized ALG-CHO-Catechol hydrogels, (d) PLGA/ALG-CHO-Catechol hydrogels.

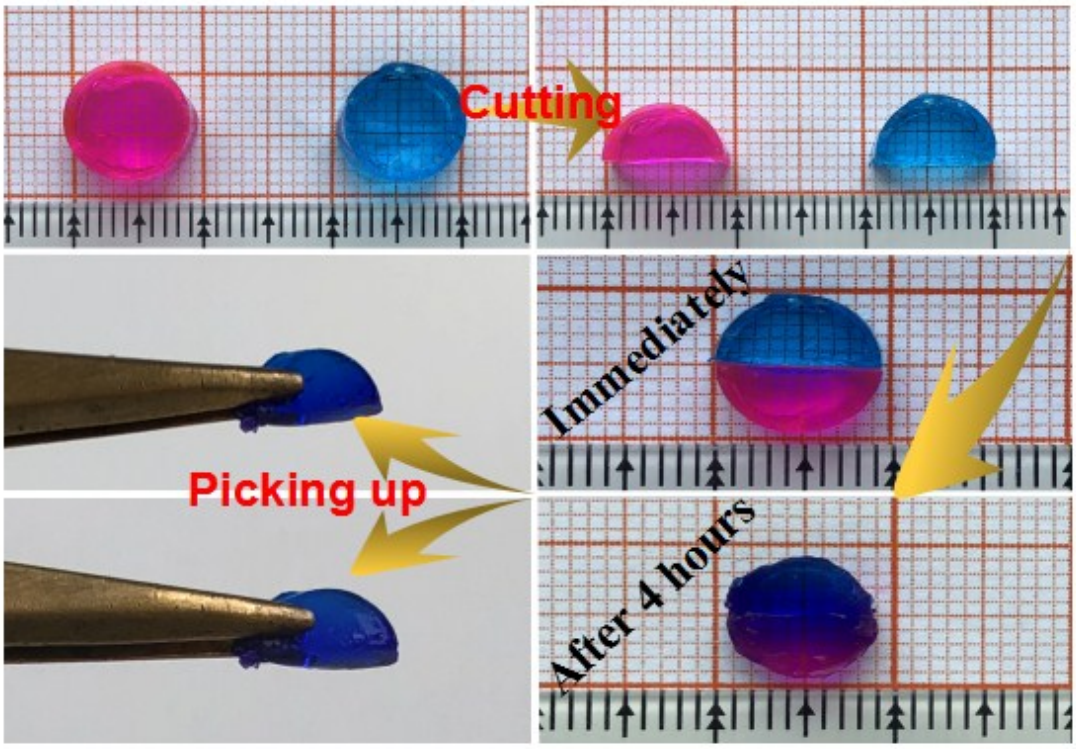

Fig. S2 Evaluation of the self-healing behavior of the PLGA/ALG-CHO hydrogels. The hydrogels were cut into halves, and then the fractured surfaces of the two different colored hydrogels were brought into contact for different times to evaluate the self-healing behavior.

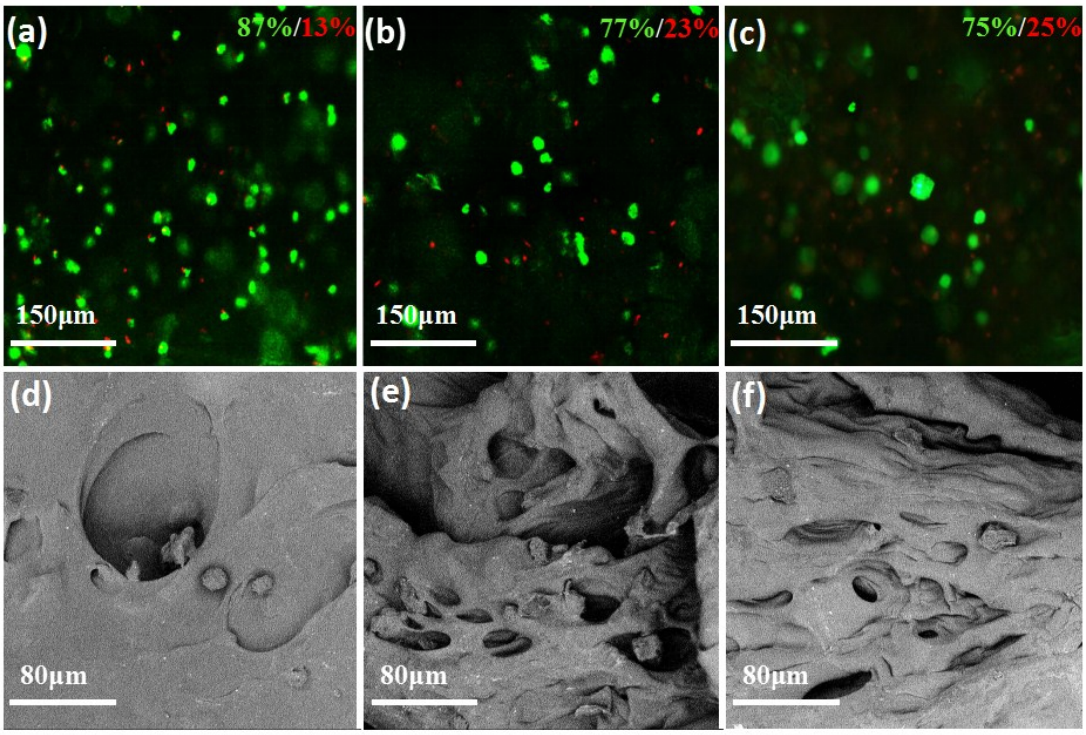

Fig. S3 Cytoviability assay. Fluorescence micrographs of the ADSCs encapsulated in at different culture time: (a) 1d, (b) 4d, and (c) 7d. The living cells were stained with FDA (green) and the dead cell nuclei were stained with PI (red). SEM images of the ADSCs encapsulated in PLGA/ALG-CHO-Catechol hydrogels after (d) 1d, (e) 4d, (f) 7d of culture.